[IMPERIAL PRIZE ESSAY.]

EXPERIMENTAL

DIPHTHERIA.

— BY —

DR. OTTO HEUBNER,

A. O. Prof. of Medicine, University Leipzig.

— TRANSLATED BY —

H. G. BEYER, M. D., M. R. C. S.

P. A. Surgeon U. S. Navy, Curator Materia Medica, U. S. National Museum.

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PREFACE.

The work which I am about to present to the medical profession in the following pages, has achieved the great honor of being awarded the prize offered by her majesty, the Empress-Queen Augusta, for the best research on diphtheria.

No one can be more fully aware than the author that he has fallen short of the end sought by the giver of the prize, viz., the discovery of a remedy for diphtheria. But in seeking for such a remedy, the following investigations had their origin.

Having lost many a battle with this disease so terribly destructive to infants, I have frequently asked myself if the received opinions upon which our present treatment is based are correct, and here I encountered grave doubts. I was at last moved to make the attempt of looking deeper into the nature of diphtheria.

But few are the steps, and limited are the results, which can be taken by a single individual, in a pursuit like this. It must be left to the judgment of the reader whether or no my researches have brought us nearer the truth, or have expanded our views of it. What I here present is merely the result of my experimental research. My views upon the nature and treatment of human diphtheria—partly the result of these experiments, partly the result of clinical thought aroused by them—I do not give here because I have not yet had an opportunity to put them into practice with that accuracy which necessarily applies to all experimental research. I reported them in brief in the Transactions of the Second Medical Congress, at Weisbaden, and refer those who are interested in them to the reports of this congress, which will shortly appear in print.*

Before one attempts by artificial means to produce in an animal, a disease which is calculated to throw light upon analogous processes in the human subject, it is necessary, above all, to first have as clear and definite a conception as possible of the human disease itself. This necessity becomes particularly apparent when we reflect on the manifold views and varying importance which have been attached to the terms diphtheritis and diphtheria, by individual medical authors in the last few decades.

This denomination, which, since Bretonneau's time, has been given by all physicians to an acute general disease, did not primarily originate in a desire to express the nature of the disease through which the entire organism

* See Appendix.

is involved in a feverish condition, in which the heart, the kidneys, the spleen and nervous system take part, but in the fact that a peculiar local process, the formation of fibrinous membrane on mucous and granulation surfaces, became very characteristic, and the presence of which in certain localities is alone sufficient, in a mechanical way, to terminate life.

In the course of time, however, cases occurred, in which the formation of false membranes did not constitute so prominent a character of the disease, and was not in itself dangerous to life, but in which everything pointed rather to the existence of a grave constitutional infection, involving the entire human organism in a condition of blood-poisoning; when in addition to this a number of experiments, which were made, seemed to prove that by inoculation of certain matters from a diseased human being, diseases, including diphtheria, could be transferred to animals, the importance of the term diphtheria gradually lessened, and began to be more and more restricted to the constitutional condition which always obtains in Bretonneau's disease. And when anyone now speaks of diphtheria of the cornea, for instance, no thought at all is given to Bretonneau's membrane.

It is, however, evident that both elements, the disturbance of the entire organism as well as the local process of the formation of a false membrane, must be taken into consideration as essential factors in our disease. The problem of producing an analogous disease in animals cannot be considered as solved, if the artificially produced disease in animals is only one of general infection, and in which, only exceptionally, a false membrane makes its appearance, which latter is only rarely wanting in the disease as it occurs in the human subject.

But in view of the difficulty of tracing out the causes of diphtheria we must still go a step further in the analysis of our conception of the disease. If Bretonneau's disease is—according to the views of the majority of physicians—a specific one caused invariably by the same poison, we must also take into consideration the fact that Bretonneau's diphtheria, that is, the formation of fibrinous exudation in and upon mucous membranes, does not exclusively belong to Bretonneau's disease. On the contrary, we find it accompanying a great number of diseases, which according to our clinical and etiological views, have nothing in common with genuine diphtheria. Prominent among these stands dysentery, which offers a condition of things in the large intestine, which, anatomically, represents diphtheria. We find, furthermore, genuine diphtheria of the throat in scarlet fever, fibrinous exudation in different membranes in cases of small pox, measles, in the typhoid stage of cholera, in typhoid fever, on granulating surfaces, on the mucous membranes of the bladder, in certain cases of cystitis, even on the mucous membranes of the gall bladder, etc.

From these anatomo-pathological facts we may draw the conclusion that the formation of a false membrane is not peculiar to genuine diphtheria, but that its poison, in common with different other poisons, possesses the property of producing the peculiar disease of the mucous membrane. It is, therefore, to be regarded as highly probable that the immediate causes of

the diphtheritic diseases of mucous membranes are everywhere the same, but that very different morbific agents are capable of calling into existence these immediate causes, in perhaps very many different ways.

It thus follows that the problem of experimentally tracing out the causes of diphtheritis must necessarily be along two different lines of investigation, the one of which must be directed to the conditions necessary for the production of the diphtheritic membrane, the other, to the conditions of the general diphtheritic infection; in other words, to the diphtheritic poison and its relations to the local process of the formation of false membranes. This is the method of research which has been followed out in these investigations.

THE AUTHOR.

LEIPZIG, MAY 11th, 1883.

I. The Experimental Production of Diphtheritic Disease in Mucous Membranes.

The attempt to produce the anatomical changes peculiar to diphtheria by artificial means, has, as is well known, been frequently made. After Treitz, it was particularly Oertel and Weigert, who undertook a most careful investigation of the croupous exudation which follows the application of caustics to the trachea of the rabbit, and they have shown that this artificially produced tracheal croup presents the anatomical and chemical characteristics which are found in the exudations upon the surface of mucous membranes in diphtheria. After this was done Weigert proceeded to study the processes which lead to the formation of an artificial croupous membrane, and he succeeded in clearly tracing out the various steps which are necessary to the production of this fibrinous membrane, thus creating the term coagulation-necrosis, meaning thereby a process of dying under coagulation. Although, doubtless, this was a step in the right direction, it was also true, notwithstanding the identity of the product in the animal with that of man, that the method of the artificial production of this membrane was in no wise analogous to the pathological processes as they occur in the human subject. Apart from accidental cauterizations of the epithelium of mucous membranes, by which, of course, croupous membranes may also arise in the human subject, the process of the formation of croupous membranes in man is not called forth by such injuries as the action of caustics, which may be compared to a poison possessing the property of destroying the epithelium; and the idea that perhaps bacteria exercise an analogous influence over the epithelium, remains for the present a mere hypothesis.

Thus, even after Weigert's work, there remained to be discovered a method for producing the diphtheritic process in the mucous membrane of an animal, which one might well imagine to resemble the process obtaining in the human organism. In this I was successful upon the following grounds:

Cohnheim had noticed that a temporary interruption of the circulation in certain tissues, as the ear of the rabbit, the kidney and the testicle was followed by a series of inflammatory processes, which under considerable swelling and the formation of infarctions within the tissues, finally lead to necrosis. Litten thereupon undertook the further study of these effects, which were produced by a temporary interruption of the arterial blood stream upon the tissues of the kidney, and observed among other results, that the epithelia of the kidney had undergone the same peculiar process of dying which Cohnheim and Weigert designated coagulation-necrosis.

If such an effect could be produced upon renal epithelia, it was also to be supposed that the pathological process which is indicated by the term diphtheria, and which is nothing else than a coagulation-necrosis of the epithelium of the mucous membranes, and even of the mucous membrane itself, might also be produced, under analogous conditions, on mucous membranes.

Diphtheria being nothing more nor less than such a coagulation-necrosis of mucous tissue, it was to be supposed that the mucous membrane, under analogous conditions, would undergo the same peculiar changes as the renal epithelia.

This supposition was fairly realized after the proper object for experimentation had been discovered.

For, above all, it was necessary to find a tract of mucous membrane to which this method could be applied, and in which it was possible that the part could be shut out from the general circulation for a given time. It was hopeless to entertain such an idea with regard to the mucous membrane of the throat. The uvula would have answered the purpose very well indeed, but neither dogs nor rabbits possess uvulæ. Some experiments made on the third tarsal cartilage of the rabbit failed to give any clear results, probably on account of the interruption of the circulation not being complete.

At the suggestion of Professor Cohnheim, to whom I am very much indebted, I chose the urinary bladder of the rabbit. This organ answered our purpose completely. The fundus of the bladder of the rabbit is supplied with blood by two arteries, which run up on either side from the neck; the blood is returned by veins pursuing a similar course to that of the arteries. If we surround the bladder of the living animal with a ligature, in such a manner that

the ligature will come to lie near the neck, if possible above the opening of the ureters, the circulation in the fundus of the bladder will be completely arrested. For this organ, which projects into the abdominal cavity, being covered by peritoneum, does not receive any blood supply in a collateral way, and a sudden arrest in the circulation after the above method, is brought about. If the ligature is removed after a certain length of time, the circulation is restored in those vessels which are free from thrombi.

By this method we do not produce the so-called anæmia or ischæmia, for by means of the ligature the blood-current in both veins and arteries, is stopped at the same time. What really takes place is the sudden arrest of the entire blood column contained within the ligated organ. In this the method more closely resembles that of Cohnheim than that of Litten. A certain share of what happens, after the ligature has been removed, must necessarily be ascribed to the influence of the nervous system in these as well as in Cohnheim's experiments. What that share is has already been mentioned, in a very clear and concise manner, by Cohnheim.

This complicating circumstance need not be taken into consideration, inasmuch as it is the immediate conditions, not the remote ones, that determine the affection of the mucous membrane, which we are trying to study. At any rate, if it succeeded in producing, in this manner, the anatomical conditions of diphtheria, a method is found by which the portion to be experimented on remained perfectly intact, and is subjected to none but functional disturbances in such a way as they may well occur in the human subject. We will see from what follows that this proved surprisingly successful..

The operation is simple, and must be made under antiseptic precautions. The rabbit is tied with his back to the table; an incision about three or four centimetres long, and beginning immediately above the symphysis is made in the linea alba. The extent of it must be just sufficient to admit of the protrusion of the bladder, which is generally filled ; it must not be larger, in order that the protrusion of the intestines and uterus may be prevented.

The fundus of the bladder carefully drawn out, under a constant carbolic acid spray, is quickly ligated, and returned into the abdominal cavity. Everything is done in a few minutes. The wound is then closed with several sutures, one end of the ligature, however, protruding from the wound in order to facilitate the second

step in the operation, which is to cut the ligature without being obliged to draw the entire bladder out from the abdominal cavity. After this the skin of the abdomen is sewed up, and the animal set at liberty.

At the end of two hours—the uniform duration of the arrest of the blood current—as many sutures are removed as are necessary for the removal of the ligature; the ligature is pulled out, and now the abdominal walls are most carefully closed with numerous sutures, under the carbolic acid spray.

If this operation is carried out under antiseptic precautions, it will be very successful and the wound will heal without the formation of any pus. It will, however, in some cases, happen that a slight pus-cavity forms between the skin and muscle of the abdominal parietes.

In two out of twenty-five operations septic infection occurred, and in both instances I had been assisted by a friend who was doing a great deal of dissecting at the time.

I will now give a detailed description of the experiments:

EXPERIMENT I.—A very strong rabbit was operated upon on November 23d, 1880, in the manner indicated above. The ligature around the fundus of the bladder was kept on for two hours, and thus complete arrest of circulation brought about for that period.

On the following day the animal was very well, ate well and had no fever, the temperature being 38.9° C. About 40 c. c. of urine which was collected gave 1-5 vol. albumen, and contained some blood corpuscles, epithelia, granules, etc. No pus.

November 25th. Temperature 39.6° C. Animal ate well; copious movement of normal stool. Urine about 10 c. c. with 1-4 vol. of albumen; is of darker color, contains peculiar sharp-edged epithelia not unlike those which are characteristic of diphtheria, epithelia containing blood pigment, blood corpuscles, some leucocytes, mucus, and a finely fibrinous coagulum within which are enclosed several blood-corpuscles.

November 26th. Temperature 39.5° C. Animal ate but little, drank a great deal, otherwise general health seems good. Urine collected amounted to 25 c. c., contained 1-3 vol. albumen, much mucus, a few round cells, and clusters of epithelia. Some very well-preserved epithelia hang together with some peculiar non-nucleated "schollen."

At 3:30 P. M. on same day, that is, at the end of the third day after the operation, the animal was killed with chloroform.

The post mortem was made immediately.—Between skin and muscle of the abdominal wall and near the seat of the operation, a small, flat pus-cavity was discovered. Muscular walls healed perfectly. Peritoneum shows no trace of inflammation. Anterior wall of bladder sticking loosely to abdominal wall; no pus.

The fundus of the bladder is of a dark-red appearance externally, very much swollen, its walls considerably thicker and stiffer. On opening the bladder, the mucous membrane above the ligature, shows marked swelling, a dark-red discoloration, and is dotted all over with ecchymotic spots; contrasts very strongly with the part below the ligature.

On close examination and after spreading out the entire bladder fundus, it was found to consist of hyperæmic, hæmorrhagic, and markedly swollen tissue. Within this region, however, we find a small scar of the size of an old three-penny piece, which shows much greater swellings and by its yellow color is easily distinguishable from the rest of the surrounding membrane, macroscopically presenting a picture very much like what is seen in a diphtheritic patch. Only a very small part of the fundus, to the extent of 1-2 sq. cm., is a yellow, firm piece of necrotic tissue.

Thus, it was found, that by an arrest of the normal functions of the circulation in the organ, we had produced a condition of things, much resembling that of a diphtheritic exudation. Before we now enter into the histology of the resulting tissue, we propose to follow out in more detail the chronological sequence of the entire development of the process and to give for each one of the different stages, both its macroscopical and its microscopical appearances.

(*a*) If an animal, upon which the above operation has been performed, be killed within the first twenty-four hours, or towards the end of the first day after removing the ligature, the bladder presents the following characteristic appearances:

That part of the bladder which is immediately below the ligature is intensely injected; this extreme vascularity gradually fades away towards the mouth of the urethra. In the part above the ligature necrosis is observed to have set in at points, extending to the entire thickness of the wall, where the circulation has not been restored. In very successful cases, however, no necrosis is generally found, or

exists to only a very slight extent. The larger part of the wall of the bladder is considerably swollen, and has sometimes a reddish-gray, sometimes a dark-red hæmorrhagic appearance. This swelling is mainly due to an œdematous condition of the submucosa, the mucous membrane itself having actually no room for accommodating itself to this changed condition of things and is thrown into numerous folds and rugæ. If the bladder is left to itself a certain length of time, these folds, rugæ and elevations of the mucous membrane, go down and eventually disappear, a great deal of fluid, at the same time oozing from the edges of the incision, thus proving that the swelling is due to an accumulation of fluid within the walls of the bladder. If the bladder in this condition is placed in absolute alcohol or is boiled, the fluid is retained and the exact seat of it can be studied ; this of course cannot be done without the occurrence of a good deal of shrinkage. Very fine pictures are also obtained from frozen sections.

In very fine sections the following may be made out: The thickness of the bladder wall has increased from five to even ten times its normal dimension. The principal part of this thickening is found in the submucosa. The meshes of the submucous layer are distended with fluid and the mucous and muscular, as well as serous layers, are also in a succulent, loose condition. The fluid, after it has undergone coagulation by absolute alcohol, exhibits a very fine fibrous network with more or less larger and smaller meshes. By boiling according to Koch's method, this network reveals itself as a homogeneous substance causing slight refraction of the rays of light; in frozen preparations it has a granular appearance. It is easily and very intensely stained by eosin. It is obvious, then, that we have here to do with a serum rich in coagulable albumen.

The bloodvessels (veins and capillaries) throughout the entire bladder, more particularly in the mucosa close up to the epithelial covering, and also in the serosa, are very much distended and crowded with blood corpuscles. In a number of places may be seen a marginal zone of white blood corpuscles, which like a wreath surrounds the lumen of the vessels. All along the bloodvessels and also at a certain distance from them, there can be seen an extra-vascular cell infiltration which is very dense in places where the tissue is less œdematous, and less dense where the œdema is more marked. The nuclei of these cells, which are apparently nothing

else than white blood corpuscles, are everywhere perfectly stained, even in places where the œdema is most pronounced. But the red corpuscles have also escaped from their vessels in great numbers. They lie scattered in the most superficial layer of the submucosa, in the mucosa, and to a certain extent also in between the epithelial cells partly in the form of ecchymoses and hæmorrhagic infiltrations, and partly as single cells.

More particularly interesting is the appearance of the epithelium of the mucous membrane itself, which, normally, in the rabbit also, is a stratified epithelium. It is distinctly loosened in its connections and the most superficial layers have become distinctly swollen and enlarged. A few of those swollen cells contain vacuoles and others present a kind of corroded appearance, somewhat after the manner that Wagner has described as fibrinous degeneration of the epithelium, but wanting in the peculiar lustre of the prickles. Such formations are particularly apparent in boiled preparations The albuminous fluid between and on the surface of the epithelial cells is coagulated and intimately adheres to them. Such masses are stained uniformly red in eosin, but nuclei can no longer be distinguished in them, and even in the main mass of epithelia the nuclei are only stained with difficulty.

Fig. 1, right half, and Fig. 2, represent pretty well the appearances just described. Fig. 1, is from an animal killed on the second day after the operation ; and Fig. 2, from one killed on the first day. The latter preparation is taken from a piece of boiled tissue. The staining is with eosin and gentiana violet. In Fig. 1, one can recognize the loosened and œdematously enlarged epithelial cells with faintly stained nuclei among the mucous and submucous layers in like condition. In between them there are hæmorrhages proceeding from a neighboring bloodvessel.

Fig. 2, represents a portion of epithelial tissue under a stronger magnifying power, with effused liquid between the cells.

(*b*) If the animal is allowed to live somewhat longer, say from thirty to thirty-six hours, we find essentially the same condition of things as in the previous case. But now a certain number of little swellings can be noticed, generally of a red or reddish-black color, which do not collapse when the watery fluid is allowed to run off from the wound of the incised bladder. These swellings consist, therefore, of a congealed exudation. The histological in-

vestigations of such places show that they consist of an accumulation of homogeneous masses, and partly also of the debris of white blood corpuscles, but for the greater part are composed of red blood corpuscles, in the mucous as well as the submucous layers. Staining with Bismarck brown or gentian as violet is unable to reproduce the original connective tissue framework in this mass of homogeneous infiltration, but everywhere we find exudations through which can be traced aggregate masses of detritus in the place of the former connective tissue.

(Vide Fig. 1, left half. In it one recognizes that the entire mucous membrane, including submucous tissue, has been transformed into a substance which is stained red with eosin; scattered rather uniformly all through it, there is a rich nuclear detritus which is stained blue, and which contrasts beautifully with the right side of the picture in which the original normal structure is retained. It is a complete diphtheritic patch.)

The epithelium is wanting in those consolidated little swellings above mentioned, or has become resolved in them. On the whole it is still present even here, but all the cells are swollen up. The nuclei of the superficial layers take but a very faint stain or are only partially stained, and have a folded appearance. The protoplasm of the cells contains vacuoles and is shaggy. All the rest of the tissue is as in the case (*a*).

(*c*) If the animal is kept alive forty-eight hours after the operation, one perceives between the hæmorrhagic, œdematous swellings which cover the entire mucous surface, some few small patches of a yellowish color and quite firm. These are found sometimes in the neighborhood of the place of the ligatures, and sometimes they can be more easily followed towards the fundus and can always be differentiated from the surrounding portions by their color and peculiar lustre. The yellowish patches consist uniformly of rigid, congealed, dead tissue, as in the second case, only that the discoloration is still more marked. Nowhere can a single definite nucleus be found, and even the nuclear detritus is but very scanty. There is no trace of any epithelium to be found on the surface. The tissue can be stained intensely red with eosin. Nearer the muscularis a larger quantity of stainable nuclei may be found. These cells, however, come from the dilated vessels of the submucous layer, which like a wreath surrounds the dead tissue.

In this stage one can study the origin of such patches in a few places. Here and there is a narrow zone of submucous tissue without nuclei, and rigid. When such a zone is followed out one suddenly comes upon a very copious extravasation of red and white corpuscles, surrounding some still well preserved dilated blood-vessels. This extravasation can be followed downwards into the deeper layers of the submucosa, and upwards into the deeper epithelial layers, thus forming a sort of biconcave infiltration on the borders of the mucous membrane.

The epithelium, when it is still well enough preserved, on those portions, for example, which have not suffered too much, still shows a copious œdema; every single cell is twice or three times larger, every still stainable nucleus is surrounded by a light halo, which separates it from the surrounding protoplasm. Here and there the upper layers of the epithelium have fallen off. Frequently dense infarctions of the entire epithelial stratum into blood corpuscles are met with, and also in certain places a congealed mass is found.

(*d*) If the animal is killed seventy-two hours after the removal of the ligature, we meet, macroscopically, with the picture already described in connection with Experiment I., and know now that the transformation of the mucous surface into a rigid, pale yellow, voluminous mass, found now more extensively, is nothing else but a far wider extension of the coagulation-necrosis of the epithelium of the mucosa and sub-mucosa, which already commenced on the first and second day. For, if we again examine these infiltrations of the mucous membrane, which resemble diphtheritic patches, we meet here, as well as elsewhere, with the same structureless, rigid, dull-shining mass of tissue, deeply stainable in eosin, but in no wise showing the presence of living nuclei. The only difference that exists is one of extent in depth as well as in breadth, but not in kind; and now we find that not only the mucous and sub-mucous layers are implicated, but also the muscular and serous layer of the bladder. Here and there it is easy to trace the red blood corpuscles, and their part in the production of this congealed substance, by the peculiar color. Also, nuclear detritus and secondary cell heaps are occasionally met with among these patches of congealed substance.

Beside those diphtheritic portions of the bladder-wall, we find

still large territories occupied by an œdematous hæmorrhagic infiltration.

There are now also changes noticeable in the epithelium, of a much greater extent than in previous stages; changes by which we can plainly recognize the transformation of living cells into a diphtheritic and croupous membrane. If one traces out the epithelial layer on one of the sections comprising a very large portion of the affected mucous membrane, and notices as it passes after the more or less affected portions, then he finds that the epithelial layer, all along its entire extent, has become changed into a homogeneous, uniform, narrow zone or lamella, which still adheres to the mucous layer. The mucosa underlying such lamella is at times infiltrated with cells, and at times, already, no trace of any nuclei can be found; such places, in which both mucous and sub-mucous layers have become transformed into a diphtheritic patch, are very convincing of the fact that the upper zone of it must have been produced by the transformation of the original epithelial layer into a homogeneous lamella.

In figure 4, for instance, that portion of the diphtheritic scab which is situated between the upper bluish-red line on the surface, and that brownish-red stripe below (due to a hæmorrhagic infiltration), is transformed epithelium. Of this it is easy to convince one's self, and all that is necessary to do is to trace this line to the margin of the scab, where one will arrive at a point at which this layer will gradually verge into one of still plainly recognizable epithelium.

In other places we meet in place of the epithelium, sometimes homogeneous, sometimes a more or less firmly reticulated structure heaped up considerably, and exceeding by far the natural thickness of the epithelial layer. In some more fortunate places one finally sees how a still preserved epithelium can be seen passing into a heap of irregularly shaped lumps, whose shape still reminds us of that of epithelial cells, but whose nuclei have ceased to be visible, and closely adjoining it we once more find an amorphous or fibrous substance representative of the membrane.

In figure 3, we have pictured a croupous membrane occupying the place of the epithelium, and resting upon the mucous layer, which shows a very abundant nuclear infiltration.

(*e*) Almost the same description as that just given of the appearances of the mucous membrane of the bladder in the previous

stage of disease, would also answer for the appearances presented by the bladder of an animal killed at the end of the fourth or fifth day of the operation, only the coagulation-necrosis becomes the most prominent characteristic, while the hæmorrhagic infiltration almost disappears.

Before pursuing further the process of the disease, it would, perhaps, not be useless to devote a little time to the consideration of the anatomical picture of the disease which has been gradually developed before our eyes.

Did we have a right to use the word "diphtheritic" in the above description of the condition of things as they presented themselves—have we here really to deal with a disease of the mucous membranes which resembles human diphtheria? This question can, perhaps, without reserve, be answered in the affirmative, so far as concerns the morphological identity existing between the local process of the disease as it occurs in man, and that which has been experimentally called into existence in the bladder of the rabbit. Let us first consider the changes which we have observed in the epithelium, and we find on the beginning an œdematous swelling and hæmorrhagic infiltration; then follows, under the development of peculiar changes in shape, which we know from a description of human diphtheria many years since,—the death of the cells, the transformation and metamorphosis of very extensive territories of the epithelial stratum—into lifeless masses; and next the new formation of a fibrinous membrane, formed partly from the dead-cell-material, and partly from material furnished by the extravasated blood constituents. This is evidently the same process which underlies the production of the diphtheritic croup of man, and as it has appeared more clearly from the later investigations of Weigert. In the majority of cases of human diphtheria the process is limited to this class of croup. The latter, indeed, as Weigert has ascertained, is anatomically in the throat, larynx and trachea, as a mere superficial coagulation-necrosis, with the products of coagulation super-imposed, not interposed, and is therefore called pseudo-diphtheria.

In those experiments of ours, we find, however, more than a mere superficial diphtheria; we find a tissue-diphtheria. The tissues of the entire mucous and sub-mucous membranes have become changed into diphtheritic patches; it dies and swells up on account of its having become infiltrated by a coagulable fluid material. This

death of the tissue by coagulation under an increase in volume, is not generally met with to the same extent and in so many cases as it has been found in our experiments; but it is met with very extensively in human pathology, where we, with perfect right, speak of genuine diphtheria, namely, the diphtheria of scarlet fever and epidemic dysentery. Our experimentally produced diphtheria, indeed, resembles much more the processes of the production of the latter two than those of the products of primary human diphtheria.

This circumstance, however, will be at once understood when it is remembered that we were obliged to put the entire bladder wall and not only the most superficial epithelial layer simultaneously under the same influence and under the same condition; and, therefore, we are still free to believe that an isolated influence of the mucous membrane would also have produced an isolated case of disease and such would be identical with that same disease of the mucous membrane which we meet with in primary human diphtheria. That this really does succeed under certain circumstances will be shown later on.

If this is accepted, it follows that the experiments above described point to a new and certain method by which the diphtheritic process of mucous membranes can be artificially induced in the animal; this method has the advantage over that hitherto in use, in that the result is produced not by a direct lesion of the diseased mucous surface, but that the disease which is induced is brought on from a distance, and in this respect the method of artificial production resembles much more the pathological process necessary for the production of diphtheria in the human subject.

But still it would, after all, not amount to a great deal, if it did not at the same time give us an opportunity of following out the development of the entire process in a much more exact manner than it has hitherto been possible to do, if it did not present us with new features relative to the immediate causation of diphtheria.

And now, therefore, we must analyze and find out what it is that really happens when we ligate, *en masse*, an organ provided with a mucous membrane.

The two most essential results of this ligature around the entire bladder are the following:

1. The blood in all the arteries, veins and capillaries, in fact, the blood contained in the entire vascular tree of the organ, stagnates

for the period of two hours; or in other words, the velocity of the circulating blood in all the branches of the ligated arteries and veins = o.

The tension of the tissues need, therefore, not necessarily experience or be subjected to any alteration, because nothing can be admitted and nothing flow off; all movement ceases and the only change which can possibly take place is in the normal processes of diffusion between the contents of the blood-vessels and the fluids outside of them, but even this only takes place to a limited extent, since the chemical composition of the fluids during the two hours the ligature is on, is not essentially changed. Or, shall we accept the interpretation to the effect that, during this time, decomposition takes place, and that the products thereof are diffused through the coats of the blood-vessels and prove hurtful to the tissues? This interpretation has been rendered improbable, if not impossible, by an experiment of Cohnheim, who has proven the fact that the same kind of a process of inflammation is produced in any vascular district in which the vessels have for a time been ligated, and the blood having been substituted by salt solution; so that whether stagnated blood or a salt solution fills the vessels, the result of readmitting the normal circulation is the same, and therefore decomposition of the circulating media can have but little to do with it.

But, and this is the most important thing for the understanding of the entire process, the stagnated blood within the vessels remains in a fluid state; it remains transportable. The column of blood, on removing the ligature, is again moved on, fresh blood re-enters and circulates through the vessels in which before it had been stagnated. If, however, it does happen, as, indeed, it sometimes will, that coagulation takes place, then a simple tissue necrosis is the result. One of the requirements, therefore, is that the vessels remain open.

2. The entire mass of tissue of the bladder included in the ligature, and not excepting the coats of the blood-vessels, is deprived of its normal nutrition for the period of two hours. But in a short time, the tissues will commence to absorb oxygen from the blood contained in the capillaries, and give off carbonic acid instead; they may even receive albuminous material from the plasma, by diffusion; but soon everything of that kind must cease, until a renewed afflux of blood takes place.

Whether any abnormal conditions of excitation are caused by any injury, done to ganglion-cells in the process of ligating, such for instance, as contraction or other nervous influence, remains unknown, and may fairly be disregarded for our purpose.

That very great alteration of tissue, during the time of the ligature being in place, does not take place, can easily be demonstrated by comparing the exterior of the bladder before ligation, with the time when the ligature is about to be removed; no change in appearance can be noticed.

Let us, then, consider the second head and ask ourselves how far the production of diphtheria is influenced by the direct consequences of the deprivation of the tissues of their normal nutrition for two hours, and we receive a very precise answer, indeed, from our experiments, in studying the behavior of the epithelium after the ligature. Epithelial cells, sooner or later, but inevitably at the end of a certain time, pass through a series of changes which terminate in the death of the cell; and this death must be induced by the lack of nutrition, in fact, inanition brought about by the ligature. For the same thing cannot be explained by the processes which are in action after the ligature is removed and the normal circulation has been re-established. The most copious transudations of blood, serum of blood, and lymph corpuscles upon the mucous surface, are entirely unable to bring about death of the epithelia, a fact which is well-illustrated in many cases of catarrhal affections, in broncho-blennorrhœas, in gonorrhœa and the intense hæmorrhagic œdemata affecting the conjunctivæ and lungs. In all these cases, even if they last many hours, the epithelia of the affected mucous membrane remains, as a general thing, alive. We are, therefore, compelled to look upon the ensuing necrosis as a thing by itself, and as directly produced by the blood stagnating, and not by the consequent inflammation which ensues on the re-admittance of the normal blood-current. Also Litten looks upon the necrosis and death of renal epithelia as due to arterial anæmia of the organ. Consequently, what is true in the case of epithelia, what we are thus enabled to study step by step on epithelia, must also be true for the rest of the tissues under the same conditions. The cells of the connective tissue corpuscle as well as those of the muscle, show a gradual disappearance of their nuclei and a paling of their normal contour, until a complete dissolution of all and every kind of tissue has taken place.

Even the walls of the vessels undergo an analogous change; all the constituent elements, endothelia and muscular tissues are exposed to the same condition of inanition and suffer the same consequences of that condition. The blood, therefore, which circulates through them after the removal of the ligature will circulate in an abnormal system of vessels, and therewith we have the second element which plays a most important rôle in the causative production of artificial diphtheria: the so-called inflammatory exudation.

Cohnheim has already sufficiently established the fact, that the interruption of the normal blood-current produces a lesion in the walls of the blood-vessels, a molecular alteration as it has been called lately, the consequence of which is a greater liability of these walls for letting their contents pass through them. The most superficial examination of our experiments shows that these results are thereby confirmed. The large number of minute and large hæmorrhages into the tissues, the marginal position of the white blood-corpuscles in the veins, and finally the enormous œdema which every time make their appearance at the end of the first day, all this proves conclusively, that a change has taken place in the walls of the vessels which tends to produce a copious infiltration into the surrounding tissue, of serum and colloidal material through the coats of the blood-vessels.

In the interpretation of this peculiar alteration brought about in the vessels, we can now go a step further than Cohnheim. From an analogy with the rest of the tissues we may draw the conclusion, that this peculiar alteration is nothing else than the commencement of the death of the vascular walls, the beginning of a profound disturbance in the nutrition which likewise terminates in the death of the vascular walls, or at any rate may do so. For, of course, it must be admitted that, as in the case of the epithelium, the vascular walls may recover from this disturbance of nutrition and return to the normal condition. We may even go further and affirm that the vascular walls offer much more resistance to the disturbance of nutrition than any of the rest of the tissues. Nevertheless, the inherent relation of both these conditions remains, and we may, perhaps, look upon the inflammatory alteration in the vascular walls as the first stage in the process of dying.

It is of the greatest importance in diphtheria that, for the beginning of the process, the complete death of the walls of the

blood-vessels is prevented, for in such a vessel the blood would coagulate, and that nothing else but a profound alteration in the coats of the vessel is present, into which blood now flows under the normal pressure. With the readmission of blood under normal pressure begins the exudation of the blood constituents, which process forms the second factor in the production of death of the tissues by coagulation.

For if we take into comparative consideration the relation of the exudation to the diphtheria, which is in consequence thereof produced, we see that just in these places, where the disease of the walls of the vessels, with its consequences, is the most prominent, there diphtheria also is most pronounced; that these œdematous swellings give rise to diphtheritic patches, yea, that in many places the origin of a cellular hæmorrhagic infiltration can be directly traced to coagulation. It results therefrom, naturally, that this second factor is rather the more important in the process of the formation of the diphtheritic patch. But at the same time it is obvious that it is conditional, that the complete death of the vascular walls is prevented. For when this happens—and examples of that kind were very numerous in the course of my experiments on certain parts of the bladder wall—the result is simple unmodified death of tissue, but not coagulation-necrosis, and already at the end of the first day there will be found in these respective places when that has happened, a thin, dry, parchment-like, pale-yellow skin, within which no trace of any swelling can be found. It is certain, then, that the vascular walls must remain alive where diphtheria is expected; but just as important as the remaining alive of these walls, is also the fact that they must not remain normal. For without the inflammatory change—this also is well illustrated in some of my specimens—the blood-vessels likewise become distended with blood on removing the ligature; but this will not be sufficient to bring about this copious exudation, which we invariably find in the diphtheritic portions. Without disease of the walls of the bloodvessels no swelling of the portions afterwards coagulating would occur, and no diphtheritic patches be produced. On this point I cannot agree with Litten, who believes that the normal transudation of lymph from normal bloodvessels is sufficient to produce coagulation necrosis. His method of experimentation is, however, different from mine, and, therefore, a direct comparison of the results obtained by both

methods cannot very well be made. His coagulation-necrosis did not attain the extent that mine did.

Then again, the mere disease of the walls of the blood-vessels is in itself not sufficient to cause coagulation-necrosis or diphtheria. If this were the case it would hardly be intelligible why it could not be produced immediately after the removal of the ligature, or at least in a few hours after a sufficient quantum of blood constituents has made its way out of the vessels into the surrounding tissue. We find, on the contrary, as a rule, that up to the end of the first day, it is wanting, and that during the course of the second day we note the beginning of its development. But it usually makes its appearance when the necrosis of the epithelia, and that of the tissue immediately surrounding the diseased vessels, is complete. We have in the behavior of the epithelia, shown how this death or process of dying, is gradually brought about, and how, furthermore, soon after death, also coagulation-necrosis appears, and in place of epithelia fibrinous membranes arise. We must imagine the method of production in this manner: the tissues at the moment of their death give off a peculiar fibrino-plastic substance, in order being surrounded and completely permeated with fibrogenic blood plasms, to produce a coagulation material which has a much greater volume than was possessed by the original tissue. This permeation with fluid is caused by that copious inflammatory exudation, and, in the further course, these transuded blood constituents share in the formation of the constantly increasing extent of the coagulation necrosis as long as circulation exists.

So we see it now demonstrated and proven that the definition of diphtheria which was given by Cohnheim is correct, and that it consists in a very intimate relation between inflammation and necrosis.

It is to Weigert, who took the first step in the right direction, that we owe a correct explanation of the entire process. He discovered that it was conditional on the production of a croupous coagulation on any mucous surface, that the necrosis of the epithelium had preceded it; that, however, at the same time the mucous and submucous connective tissue remained undestroyed; in other words, that the circulation of the blood in those vessels adjacent to the surface remained intact. But it was not very well explained why it was that such a copious transudation of lymph and lymph-corpus-

cles should take place, in order to give rise, under the circumstances, to such fibrinous thickenings. This point has, however, become explicable even in the face of his experiments. For the action of no caustic, whereby epithelium is destroyed throughout its whole thickness, will necessarily limit its action to the epithelium, but will produce a simultaneous destructive action, at least, to the walls of those vessels of the mucosa which are situated near the surface. It is just in view of this that those volatile caustics, such as ammonia and carbolic acid, produce the best croupous membranes. The fact that a mere tissue-necrosis, the vessels and normal lymph-stream being intact, cannot produce a coagulation-necrosis that is diphtheria, is pretty well established and proven, according to my opinion, by cauterization of the central portion of the cornea. If the centrum of the cornea is carefully cauterized, no change in the remote blood-vessels of the conjunctiva occurs; a circumscribed necrosis of the epithelium is produced, in the neighborhood of which lymph circulates undisturbed; but a diphtheritic membrane cannot be produced in this way.

It must, necessarily, be pre-supposed that in all experiments at the time, when, in consequence of the necessary steps of the operation, the epithelial tissues had died, the blood vessels, although much altered, were still alive, and circulation of blood within them and transudation of blood constituents from them into neighboring tissues were still possible. This admits of direct proof.

During some other experiments which I made for quite a different purpose, I stumbled upon a method by means of which it is easily possible to produce a very fine self-injection of the blood vessels, and one that only becomes complete and perfected within the last few hours of life. When an animal is inoculated with bacillus anthracis, a certain time always elapses before these bacilli have multiplied and have become developed to such an extent that the entire vascular system has become filled with them. As soon as this has happened, life is, of course, impossible, and death supervenes at the end of a few hours. These bacilli can, by properly staining them, be traced out distinctly, and in thin sections not one need escape the eye of the observer; and thereby we have a means at our hands by which it is easily possible to follow out the course of the blood-vessels, even such as have not a single corpuscle left in them.

By means of this method, when inoculation and ligating are

properly combined, we can show that even in those places which have already become changed into coagulation-necrosis, circulation must have existed even shortly before death, and that, consequently, exudation must have been going on.

Fig. 4 represents part of such a diphtheritic patch in which blood-vessels are still present, filled with blood and bacilli anthracis which could only have gotten there two hours before death. The only living stainable nuclei of the original tissues are those of the muscles of the smaller arteries and the walls of the veins; they have already died ; nevertheless even in these, circulation had gone on at least a few hours before death occurred.

Thus it is shown, what has already been mentioned above, that the walls of the bloodvessels exercise more resistance to the destructive influence due the interruption in the normal circulation, than the rest of the tissues, and that their cells will occasionally remain alive even in the midst of the surrounding dead tissue; and the conclusion is justified, namely: that, on the other hand, they are also capable of a much more rapid recovery, restitutio ad integrum, more especially in those places in which the surrounding tissue contains material still alive. This is of importance for the understanding of this croupous affection of the mucous membrane.

If, then, so far, we have been able to recognize and properly appreciate both conditions which are necessary for the production of diphtheria, it will be comparatively easy for us to explain the variations which have occurred during these experiments.

First, as already mentioned above, it is intelligible why in all my experiments not croup, or surface diphtheria, but tissue diphtheria (Weigert's true diphtheria) was produced. We have to deal with a diseased condition of the entire bladder wall and its constituents, consequently diphtheritic deposits must occur in all the different layers. It only rarely occurs that the fundus of the bladder which has been ligatured off is uniformly affected, some places being always more affected than others. The same was found by Cohnheim in his experiments. This may depend upon, perhaps, two things : First, the ligature may not in all cases be equally tight, or may not affect one portion as much as another and only retards the circulation in some parts, while in others it may entirely arrest it ; second, it may also depend upon the individual resistance of some animals, as well as that of some tissues. It is

easily to be imagined that certain portions of the bladder might be more readily affected than others by the same operation; that in the one the damage done is beyond recovery, in the other it is not, or that also a portion of the bladder having previously been under a more favorable condition of nourishment will be longer in dying than others. And inasmuch as these differences are again subject to two different factors, the transudation from the vessels of one and the disturbance in nutrition on the other hand, which factors are not congruent in time, it is to be expected, *à priori*, that a diphtheritic bladder may look very different in different animals. In some instances these diphtheritic patches are found situated in or near the groove of the ligature; in others remote from it in the fundus of the bladder; again in others we find a coherent territory; and in still others we come across small patches scattered through the hæmorrhagic œdematous mucous membrane. Sometimes these patches are developed more prominently in the mucous and sub-mucous layers, more rarely so in the muscular and serous layers ; in some cases diphtheritic patches are developed quickly and abundantly; in others they are scarce and develop slowly. As a general thing, however, their number and extent increase in accordance with the time which elapses between the removal of the ligature and the examination. At any rate, it is of interest, and deserves to be accentuated, that every time after one operation and treatment of the animal, we obtained that peculiar diseased condition of the mucous membrane which is constantly met with in human diphtheria.

If we imagine a case in which the causes of this diseased condition lie principally in the superficial strata and could only exert their destructive influence upon the epithelium covering the surface of the mucous membrane, while the rest of the tissues did resist, we should certainly have a genuine croup or a surface diphtheria.

A fortunate accident has given me an example of it: (Experiment VII.) On the afternoon of the 23d of March, 1881, the bladder of a certain sick rabbit was ligated. On the 26th it was inoculated with bacillus anthracis. It died on the 29th, at 1:00 P.M., in other words, at the end of the sixth day. Here I found the bladder externally very well preserved, the internal surface, however, throughout its whole extent showed the most beautiful croupous appearances. Fig. 3 is taken from a section through the most

superficial croupous layer of the mucous surface. One recognizes the enormous thickness of the croupous stratum and also the adjoining; the most superficial layer is already undergoing coagulation-necrosis with nuclear detritus.

If we now scrutinize these experimental results and look upon them in the light of human pathology, the conclusion is justifiable and almost unavoidable, that there is a strong possibility, which cannot be disputed, that diphtheria may originate in the human subject in exactly the same manner as has been shown in my experiments; that, in other words, it must be admitted that a non-infective diphtheritic affection of the mucous membrane may exist. To experiments by means of cauterization, it might have been objected, that those conditions did not answer to those which produced diphtheria in the human subject. The development and presence of such mechanical conditions, however, as I have artificially induced to produce diphtheria, are, indeed, easily imaginable in the human subject. Let us suppose, for instance, a spasm of a number of small arterioles near the mucous surface (say, perhaps, reflexly by the action of cold on the mucous surface or the general surface) lasting for the period of two hours, which is sufficient to produce an arrest of the circulation in at least the remotest capillaries, that is, those situated nearest the surface; supposethis condition of things should be followed by a reëstablishment of the circulation, and it cannot fail that in such cases at the end of one and a half to two days, without the aid of a single micrococcus a croupous exudation is produced. That such a spasm of the blood-vessels might, under certain circumstances, continue to last two hours and lead to a complete arrest of the circulation, remains to be experimentally proven; in the meantime this is still conceivable.

Or, in case that a rheumatic affection and swelling be developed along the branches of the connective tissue capsule of the tonsil, through which the blood-vessels take their course, and would give rise to compression of a number of arterioles and capillaries lasting for several hours, which again must have a temporary stagnation in the circulation for its consequence, we again would have a tonsillar croup produced in a mere mechanical way.

In the same way it is possible that, *e. g.*, a spasm in the large intestine, enclosing a very thick ball of fœcal matter, may by pressure upon the epithelial-cells produce their death and at the same

time cause disease of the superficially located blood-vessels from compression—and here again the two conditions are given for the production of exudation with coagulation. In quite a similar manner, under certain circumstances, the pressure produced by a gall stone upon the mucous surface of the contracted gall ducts, etc., might produce similar results.

In short, the causation of croupous or diphtheritic disease of mucous membranes in the human subject, through mechanical interferences, is explained by the above experiments, and its independent occurrence rendered probable. It is just this which is of so much importance in (clinical) croup and (clinical) diphtheria. Those who, *à priori*, deny the possibility or probability of a genuine croup being produced without a diphtheritic poison, are welcome to dispose of the above facts.

Finally, we must direct attention to the fact, that the form and site of the coagulation-necrosis depend not essentially on any inequality in the causes underlying them, but simply on the place whereon they may find a suitable point of attack. The process of the production of Weigert's pseudo-diphtheria, or croup, is, then, essentially the same as that of genuine tissue diphtheria, which latter is beautifully illustrated and developed in scarlatina, and differs from it in only this respect, that in the latter only the epithelium and superficial capillaries are affected, while in the former, both mucous and submucous layers, including the blood-vessels, are implicated in the production of coagulation-necrosis.

There is, however, one very important circumstance, which seems to distinguish the two pathological processes as going on in man in croup and diphtheria, one from the other; it is just this which, especially with respect to the therapeutics of the pseudo-diphtheritic diseases of the larynx and trachea, plays such an important rôle. This is the well-known fact that in man, after the croupous membrane has been thrown off, a new one is formed in the same place, while in genuine diphtheria, loss of substance and ulcer will be the result.

How is this to be explained? Let us look once more at the conditons which are necessary to the production of a croupous membrane. From what has been said before, they will have to be looked for, first, in the process of dying, with coagulation of the epithelium of the trachea; second, in a diseased condition of the most superficially

situated capillaries of the mucous tissue. Mark well the difference in a disease of the capillaries and veins not resulting in immediate death. This is the point of so much importance. For, what happens when a croupous membrane is thrown off? As is well known, we find just before the membrane is thrown off, between the latter and the surface underneath it, a layer or two of cells, commonly called pus. The mucous layer then, after throwing off its epithelial layer, lies still exposed with its still diseased capillaries covered only by several layers of lymph cells, as it was formerly covered by epithelium. If these causative conditions, which brought about the death of epithelial layers and the disease of the capillaries, continue to exist, it would be just what could be expected to see a new and abundant transudation of fibrinogenous substance produced, and the fibrinoplastic substance is contained in the dying, superficially, located cells. The explanation for these recurrences of new membrane is found in the fact that the blood-vessels continue in their diseased condition. As soon as they recover and become again normal, the new formation of croupous membrane will cease. The fact, however, that the walls of the blood-vessels will much easier and more quickly recover and return to their normal condition than the rest of the tissues, has already been sufficiently dwelt upon above.

It is a very different thing with the genuine tissue diphtheria. Here the diseased blood-vessels are completely surrounded by dead tissue, and here the death of tissue will eventually also extend to the enclosed blood-vessels, which is not the case in croup.

We have seen that the circulation will continue for a time in those blood vessels which are in the midst of coagulated tissues; that, in other words, death of tissue precedes that of the vessels. but eventually death of the vessels takes place, and no recovery is possible. So far, then, as tissue diphtheria extends, everything—cells, intercellular substance, vessels—all must die. After the throwing off of the mass of dead tissue an ulcer remains. And now it is intelligible why here, unlike what happens in croup, no new exudation takes place. For only so far as the loss of substance reaches, was the characteristic disease of the vessels and tissues present; beyond it the causative conditions have been imperative, and no coagulation-necrosis, consequently, has been the result. In such cases we only get simple re-active inflammation, but no diphtheria.

By means of the experimental study just described, we have been enabled to split up the local process of diphtheria into its component parts—into its immediate causative elements. And now we are justified in accepting the fact that these immediate causative conditions underlying the production of this peculiar disease of the mucous membrane, are the same in the disease affecting the human subject ; a very profound interference in the nutrition of certain tissues, mostly the epithelium and, not very rarely, in the most superficially situated blood-vessels, in the mucous and sub-mucous layers, etc. And inasmuch as in human diphtheria we have to deal with a disease poison as the primary cause of all the accompanying disease-processes, we are, furthermore, justified in concluding that this particular poison is also the ultimate cause of that interference in the nutrition of the epithelia, mucous membrane, blood vessels, etc. In what manner, however, this poison gives rise to this local diphtheria, these changes so unfavorable to the maintenance of the normal conditions in the epithelium and other tissues, is another matter, and not shown by our experiments. It is evident, *à priori*, that this can be done just as well by a direct action of the poison on the cells from without, or through the blood vessels, as also indirectly, *e. g.*, by an irritation of the vaso-motors of the parts, or in some such way.

From my experiments it has been rendered certain that the local condition, or disease of the mucous membrane, stands in no sort of causative relation to the general processes observed in human diphtheria.

For this it will only be necessary to look back upon the general condition of these animals operated on, and it will be seen that in all aseptic cases this has in no case been at all affected. The animals all behaved just as was described in the first experiment. There is no lack of appetite, no fever, the behavior of the animal remains the same; in fact, this experimentally-produced diphtheria is a local affection, and remains such throughout. This process can be followed out for several days, inasmuch as the local process here, unlike what happens in croupous affection of the trachea, does not produce death mechanically. If, then, we follow out this process somewhat further, we find the following: First, it becomes necessary now to mention what has heretofore been left unnoticed, and that is this: It sometimes happens more especially in those dead

portions of the diseased bladder which may either be simply necrotic or diphtheritic, that we find accumulations of bacteria, occurring sometimes in irregular figures within the necrosed tissues, sometimes in long, densely aggregated platoons. These bacteria consist in simple bacilli, 1.5–1.8 μ. long, and one-third as broad, corresponding, therefore, to the ordinary bacterium dermo; they are well stained in alkaline aniline colors. Be it now, that these germs get into the bladder during the operation, or be it that they were originally contained in the blood, they were, by no means, able to maintain their vitality in the living tissue, but only found a fertile soil in the dead tissue. For such accumulations are not found anywhere, where the tissues still show staining of the nuclei ; nor are they to be found within the croupous membranes, but only and exclusively in the dead tissues of the bladder wall, and in this way they serve as an indication as to the extent of the tissue-necrosis. They do not, therefore, possess the significance of pathogenic microorganisms.

The dead tissue, however, does not remain very long in *statu quo*. A molecular detritus is produced therefrom and in consequence thereof a loss of substance results, which in those places, where the disease has attacked the whole thickness of the walls, produces holes in the organ. Perforations of that kind I have observed sometimes on the sixth day, always on the tenth and twelfth day of the disease, and in animals which, up to the time when they were killed, had been entirely well and had even successfully withstood other operations as, for instance, that of transfusion. The reason why such bladder perforations have so little influence is that, simultaneously with the advancing death of those portions of the fundus of the bladder, a new formation of connective tissue takes place around them, whereby the bladder is cemented on to the abdominal wall, or loops of intestines or uterus, as the case may be. Eventually a shrinkage in toto of the entire bladder ensues; on some places we find the remnant of the dead tissue, like yellowish little heaps over the still healthy portions of the serous layers of the bladder, and the final result of the entire process is, that the animal is minus the fundus of its former bladder and instead of that possesses a connective tissue-cicatrix; otherwise, however, no damage has been done to the general health.

In order, however, to obtain still further proof of the harmless-

ness of the diphtheritic disease, several inoculation experiments were undertaken with these artificially produced diphtheritic patches.

EXPERIMENT XX.—On the 24th of September, 1881, we operated in the usual way on a medium-sized, black rabbit. Four days later it was killed, while in apparently perfect health. The portion of the bladder which had been ligated is found partly in a condition of a high degree of hæmorrhagic œdema, partly covered with a yellowish streak, by its color well differentiated from the surrounding swollen-up parts.

The bladder is distended, carefully cleaned, and a few pieces of the diphtheritic patch cut off with scissors and rubbed into wounds made for the purpose of inoculation at the root of the tails of several white mice. Another piece was taken and placed under the skin of a rabbit and kept there by sutures.

The mice experienced no disturbance, and were living and getting along well during the first week in October.

The rabbit on October 19th is getting along well, eats well, etc. At the place operated on an abscess was formed which was found on October 23d, when the animal was killed, to have become thoroughly encysted. The pus obtained from it on October 19th contained some globular and biscuit-shaped bodies ; a mouse inoculated with it remained uninfluenced by it.

And herewith it was also to be regarded as innocuous to other animals, or at any rate to a very moderate degree.

We have, then, by reason of this course of experimentation, an analysis of the causes necessary to the production of Bretonneau's membrane, but Bretonneau's disease was not, however, constructed.

Perhaps (such was the course of thought), the disease might be produced per synthesis, by inducing the characteristic diphtheritic membrane in the usual manner and at the same time adding the diphtheritic poison by inoculation. This was the object of the second part of the experiment.

II. Attempt at an Artificial Combination of Local Diphtheria with General Infection.

Before attempting a combination of local diphtheria with a diphtheritic general infection, it seems necessary to me, in the interest of a clear insight into the process which would probably be developed in an animal with both the local and general disease, to first make use of some inoculation material, the properties of which are exactly known, and the carriers of which will prevent any chance of their being mistaken for anything else, rather than to use at once the micrococci of diphtheria, which are still somewhat surrounded with a certain mystery. For this reason I chose the poison of anthrax, the bacillus anthracis, for the induction of the general affection. It is well-known that this poison produces in the rabbit a distinctive acute general affection.

These experiments had, of course, to be so arranged that both local diphtheria and general infection should operate combinedly upon the bladder—that the latter, more especially, did not begin to operate after the local process had already reached completion. This is exactly what afterwards happened in the beginning, and only after many repeated experiments did we find that it was necessary to make the inoculation somewhat early, in order to obtain a simultaneous action of both ligature and poison. It is, however, well-known, that the duration of life in the rabbit after the inoculation with bacillus anthracis is but short, and that, consequently, only the early stages of diphtheria could be successfully studied, in combination with the infection. It was just this combination which gave us very beautiful results.

The following experiment teaches us how the infection material behaves in the first stages of diphtheria:

EXPERIMENT XXXI.—On November 12th, 1881, between 2:00 and 4:00 P. M., a strong rabbit was operated upon in the usual way. Immediately afterwards a fresh crop of bacilli anthracis was inoculated into both ears. On the 12th, in the evening, the animal was still alive, but on the morning of the 13th it was found dead.

The fundus of the bladder showed a high degree of œdema, the œdema, however, flowing off rapidly from the sub-mucous spaces. Only in the immediate neighborhood of the furrow of the ligature, four or five little swellings, of the size of a pin's head, were discerned, which, after the collapse of the surrounding mucous membrane, stood out in relief as diphtheritic patches.

The histological examination showed the beginning epithelial necrosis, hæmorrhagic œdema, etc. On those patches was found well pronounced coagulation-necrosis of the tissues.

The vessels of the entire wall of the bladder were filled with bacilli anthracis. While the aggregation of bacilli in the larger veins and very wide capillaries only occupied a part of the cross section of their lumen, the majority of the capillaries are entirely filled and crowded with bacilli, so that in the course of such bacilli which had been stained in gentian blue a number of vessels became visible. Those capillaries which are situated immediately underneath the epithelial layer were particularly crowded with bacilli. In some places it even looked as if these bacilli had penetrated through the capillary walls and made their way into the epithelial layers. By the use of high magnifying powers we succeeded, however, in showing that in most of such cases we had to do with oblique sections, in which the epithelial layer happened to lie either above or below the mucous layer, in whose most superficial vascular loops a few stray bacilli were found. Pictures of undoubted proof of bacilli within the loosened epithelia, as was the case with red blood-corpuscles, I have been unable to obtain. Also in the rest of the tissues no bacilli were discovered outside the blood-vessels.

Fine sections of these rigid little swellings showed a homogeneous tissue free from nuclei and richly infiltrated with red blood corpuscles. Within such structureless masses could be seen coursing somewhat brighter channels within which were found bacilli, though not so densely crowded together. A closer examination of the boundary of channels revealed feebly stained nuclei at just about the same distance from one another as were the nuclei in the capillaries and the smallest veins.

The finished coagulation-necrosis is, then, still permeated by blood-vessels, and by means of these, the diphtheritic patch was still traversed by bacilli, although no extravasation of them had taken place.

The following experiment will show that the bacilli contained within these diphtheritic patches, still possessed the property of an infective poison:

After the bladder had been taken from the animal and been extended, it was first of all thoroughly cleansed with water, in order to remove any trace of blood which might possibly adhere to it. The mucous surface was then thoroughly rinsed off with absolute alcohol. One of the segments of the little hæmorrhagic swellings was now removed by means of a pair of well-cleaned scissors, and with the juice pressed out from the cut surface a mouse was inoculated at the root of its tail. At the end of twenty-two hours the mouse died of anthrax.

This experiment shows that an infectious poison accumulates and, possibly, increases in virulence, in the portions of the mucous surface affected with diphtheria, most of the capillaries being so crowded with bacilli, as is only likely to be the case in simple cases of inoculation. It shows further that the poison can be found in the diphtheritic patches when, at the time of the full development of the infective process, circulation is still going on within the coagulating tissue. The diphtheritic patch has then become poisonous and can be used for the transmission of the infectious disease from one individual to another. We did not succeed, however, in obtaining proof, at least so far as the bacillus anthracis is concerned, that the bacilli leave their inflamed blood-vessels, and thus penetrate into the more superficial strata of the diphtheritic membrane.

Even in a somewhat later inoculation the poison still finds its way into the patch, as is seen from the following experiment:

EXPERIMENT XVIII.—On the afternoon of September 15, 1881, a strong rabbit was operated upon in the usual manner. On the 16th, at 4 P. M., it was inoculated with bacillus anthracis. It died during the night of the 18th, or two and one-half days after the inoculation, and three and one-half days after the diphtheritic infection.

The diphtheria in this case was much more extensive, but had not developed in a very marked degree. (It had been ligated with rubber tubing.) Very fine diphtheritic patches were found at a short distance from the furrow of the ligature. Within the substance of these patches, a tissue absolutely free from any nuclei, we find some very small, stained bacilli anthracis arranged in rows. Closer observation reveals the fact that they occupied the place of a former

capillary (still to be recognized by its contour), which, before death ensued, had become resolved in the general coagulation-necrosis. The bacilli had remained alive even here in the midst of dead tissue —but, of course, death of tissue had taken place very shortly before the animal itself had died, and, perhaps, very slow and sluggish circulation must have still existed in this situation at the end of the 3d day of the disease. On the dead body, however, this place could, with great certainty, be pronounced an anthraco-diphtheritic patch.

Fig. 4, taken from an analogous experiment, beautifully represents these conditions.

At a still later inoculation, coagulation-necrosis cannot be completely established before the infectious material could reach those places, and the superficial and submucous patch had no poisonous properties in such cases.

EXPERIMENT XIX.—On September 15th, in the afternoon, another rabbit was operated after the usual manner. On the 17th, in the forenoon, and, for the second time on the 18th (successfully then only perhaps on the third day of the diphtheritic disease), it was inoculated with bacillus anthracis. It died on the twentieth (or on the fifth day of the diphtheria), doubtless of anthrax. In the bladder was discovered a well-defined coagulation necrosis. Inoculation at the root of the tail of a mouse with a portion from the surface of this diphtheritic patch gave no positive result. The mouse died eight days later, but not of anthrax.

In Experiment VII, above mentioned, when a very beautiful croupous membrane was obtained, the animal had been inoculated at the beginning of the fourth day, and died on the sixth; all perforating vessels were found to be full of bacilli, but neither in the croupous membrane nor in the upper layers of the mucous membrane itself, was it possible, in spite of the most careful search, to find any.

The synthetic experiments hitherto described have demonstrated the fact, that a diphtheric mucous membrane may take up, and store up in considerable quantity, an infectious poison, provided the latter is introduced into the circulation during the formation of the diphtheritic patch when the blood-vessels are still pervious; for it seems impossible,—for the bacillus anthracis, at least—to penetrate the diseased walls of the blood-vessels of the mucous membrane.

After having ascertained these conditions, experiments with the micrococci diphtheriæ, which are much harder to diagnose morphologically, were begun:

EXPERIMENT XXIX.—On October 27th, at 3 P.M., a very strong rabbit was operated on in the usual way; at 5 P.M. the ligature was removed. During the interval, a small quantity of a diphtheritic patch taken from the tonsil of a nine-year-old boy suffering with the disease (and who succumbed to it on the 2d of November) was introduced underneath the long fascia of the back of the rabbit; the wound was carefully closed.

On the 28th in the afternoon the animal had a temperature of 41.0. C. On the 29th, 38.4 C.; on the 30th at noon, 32.5 C. in the rectum. The animal looks unkempt, moves its head spasmodically to and fro; fore paws trembling. At 2 P. M. it died. The body was kept on ice.

The autopsy was made at 10 A.M., October 31st. The inoculated muscle presented a hæmorrhagic appearance and was partly pale red, of a colloid lustre. The whole of the sub-cutaneous connective tissue of the inoculated side extending from the back to the abdominal wall, was œdematous. Spleen 5 1-2 c. m. long, thickened, dense, partly dark blue, partly brownish-red. Intestines to a large extent empty, without being particularly injected. Trachea, lungs and liver normal. Cortical portion of the kidney hyperæmic.

The fundus of the bladder shows a certain number of yellow, firmly-adhering patches, the mucous surface between these being hæmorrhagically swollen, and partially traversed by a whitish infiltration; when placed in alcohol, the epithelium comes off in the shape of a very thin skin—post mortem maceration of those portions of the epithelium which have remained intact.

According to the results arrived at from this experiment, it was, without doubt, especially well demonstrated by the swelling of the spleen, that by the subcutaneous injection of a certain quantity of a diphtheritic mass an acute infectious disease had been developed, which in a few days caused the death of the animal.

In order, now, to further ascertain the exact relation of the inoculated poison to the mechanically produced diphtheria, whether here also, the diphtheritic patch had poisonous properties as was the case in the diphtheritic infection produced by the poison of anthrax, a new experiment was at once instituted.

EXPERIMENT XXX.—Before putting the bladder of the animal (experiment 29) into alcohol, a very small bit of diphtheritic patch was taken from it, and placed underneath the skin of the back of a rabbit at 10:30 A. M., October 31st. On the afternoon of November 2d, at 2:30 P. M. (fifty-two hours after the infection, the first animal being seventy hours after the inoculation), the animal is moribund. Temperature, 366°.

The autopsy was made at 3 P. M., immediately after death.

The inoculated muscular fascia shows numerous hæmorrhages. The long back muscle shows hæmorrhages at the place of inoculation, and extending upwards as well as downwards from it, of a peculiar pale color and homogeneous appearance. Trachea free. Lungs pale red. Numerous pleuritic hæmorrhages near the margin ofboth upper and lower lobes. Intestines empty; jejunum filled with vitreous slime; nothing abnormal about the lower part of the ileum. Spleen, six centimetres long, one-half centimetre thick, very firm and dark brown. Cortical substance of kidney darkish red, with hæmorrhages. Medullary substance paler. Mucous membrane of bladder normal. Several small masses from this (not diphtheritic) mucous membrane were removed. At 4:30 P. M. two mice were inoculated with them. Both animals remain well.

The diphtheritic patch removed from the bladder of the animal which was first inoculated had, indeed, very energetic poisonous properties, when the effect of inoculation of a small particle of the same is compared with that of matter coming from a patch of merely local diphtheria. While there only a small abscess was formed around the diphtheritic eschar, we find that here a very acute infectious disease was developed, which produced death quicker than was the case in the primary infection. But that again is due to the fact that prior to the inoculation a diphtheritic local inflammation was produced in the bladder. For inoculations with parts of the healthy bladder of the second animal remained without result. That, however, the infectious poison was contained in the blood of the second animal, we will see presently. For now the qestion arises, whether it can be proven that there was present in the diphtheritic bladder an infectious material capable of augmentation.

Fine sections from the bladder of the animal from experiment 29, where a successful inoculation had taken place, show the following:

The diphtheritic process has reached a very considerable extent, so that we find on the one hand the mucous and sub-mucous layers, and on the other the serous and neighboring portions of muscular layers invaded by the coagulation-necrosis, and the layers between these are the only ones which show still distinct blood vessels and other living tissue rich in nuclei. But here we find the capillaries and veins everywhere in a dilated condition. Within these dilated vessels we find rather densely crowded bacilli, very distinctly stained with gentiana blue, and about 0.5 μ broad and 1 μ long. They are all of a uniform size; they are partly aggregated together in chains of two or four, partly irregularly scattered about through the lumen of the vessel.

In many places we see, just as in inoculations with anthrax, the bacilli penetrate into the diphtheritic patch by way of the still pervious blood vessels. Outside these channels, of course, we find an abundance of small granules and debris of irregular shape, but the presence of the characteristic micro-organisms cannot be demonstrated. In portions of the bladder wall, which are not diphtheritically affected, but in which the disease is simply inflammatory, we come across very rich aggregations of these bacilli which have penetrated into the finest mucous capillaries running along underneath the epithelial layer, just in the same manner as we have already seen in inoculation with anthrax.

Figure 5 represents fragments of two such capillaries running close underneath the swollen and œdematous epithelium, as they appear in diffused light, and shows how very much crowded they are with these micro-organisms.

These same bacilli can be found in the vascular loop forming the glomeruli of the kidneys, but not in such large numbers as is the case in the diphtheritic bladder of the animal from experiment 29.

These very same micrococci are furthermore found in the loops of the glomeruli of the kidneys of the second animal (Exp. 30), and here they occur partly in the shape of dense colonies, completely filling the capillaries, and forming emboli partly in small scattered heaps.

The emboli of bacteria must have formed in the kidney during life, inasmuch as this animal was dissected immediately after death and its kidneys placed in absolute alcohol; and now we may

be certain that both in the first as well as in the second animal, these very bacilli were the carriers of the infectious material.

It is, then, certain that the transportation of a certain quantity of diphtheritic patch from the throat of a diphtheritic child, and subsequent inoculation underneath the skin of a rabbit, produces in the latter animal an acute infectious disease which is characterized by enlargement of the spleen, and by the presence of very small bacilli in the blood, and which disease is furthermore transmissible from one animal to another. This transmissible poison, however, is especially abundant, and stored up richly and perhaps also in a purer state, in the diphtheritically diseased mucous membrane of the animal than it was originally. For inoculation with material from the diphtheritic bladder produces death more rapidly than was the case in the first animal, while inoculation of the healthy bladder of the second animal produced no result.

We have here, then, an analogous condition of things to those which we have observed in the experiments with anthrax, the only difference being that here another poison plays the rôle which bacillus anthracis played in the other experiment.

A further series of experiments was instituted, using the slimy excretions from the throat of a child sick with scarlatinous diphtheria.

EXPERIMENT XXIV—On the afternoon of October 19, 1881, a medium-sized rabbit was operated on in the usual manner. On the forenoon of the 20th, a small quantity of a slimy mass was scraped off from the surface of the palate and tongue of a two-years-old child suffering with a very acute attack of diphtheritic scarlatina. This slimy mass was kept on a hollow ground slide, carefully covered up under a cover glass until three o'clock in the afternoon of the same day, when it was transported under the skin of the back of the rabbit on its right side. It was the eighth day of the disease, and on the ninth the little girl died. The scraped-off mass consisted of mucus, pus corpuscles, epithelia, and detritus, and contained the following forms of bacteria:

1. Short bacilli and diplococci, movable.
2. Bacilli in the zoogloea form, immovable.
3. Longer bacilli very thin (bac. subtilis), movable.
4. Medium sized micrococci, forming heaps with irregularly defined outlines.

5. Small globular micrococci in densely aggregated heaps in zoogloœa form; these heaps have all a brownish color, a pretty regu larly round contour and sharply-marked appearance.

On October 22d, in the afternoon, while taking the temperature of the animal, an abnormally pasty consistence of the fæces was noticed. Otherwise nothing particularly striking was noticed about the animal. On the 23d it was found dead (between fifty and sixty hours after inoculation).

The autopsy was made on the forenoon of the same day. At the place of inoculation was found a small abscess of the size of a bean, large, purulent infiltrations of the subcutaneous connective tissue, lungs contained air throughout, in a few places small whitish infiltrations in the immediate neighborhood of the blood-vessels were seen, and also in some places in the liver, which was considerably enlarged.

No trace of peritonitis, nor was there a trace of pus or cheesy material to be found around the bladder. Spleen seven and one-half c. m. long, extremely distended, of a dark red color, capsule strongly distended, parenchyma on the cut surface hard, very pulpy. The large intestine was full of pasty fæcal matter. The fundus of the bladder resembled, viewed externally, a very dark-red, globular body, studded here and there with whitish spots. The left ureter (ligated) was much distended, of a reddish color, as was also the pelvis of kidney. Kidney swollen and dark red. Right kidney pale, of normal size; cortical portion hyperæmic and hæmorrhagic in places. In the bladder slightly turbid urine.

Mucous membranes and entire wall of fundus of bladder swollen and strongly hyperæmic and hæmorrhagic. Over the dark red and velvety mucous surface, scattered in large numbers, we find yellowish and dark-red patches, firmly connected with the underlying tissues but reaching out above the level of the surroundings; besides these there are also a few whitish scales.

Four mice are immediately inoculated with the splenic pulp, another mouse with the blood, and still another with the pus which was found at the place of inoculation.

The animals inoculated with spleen and blood died on the third day; those inoculated with the pus, on the fourth. Besides (exp. 25) on Oct. 23rd, at 12:30 P. M., a small portion from a diphtheritic patch from the bladder was brought underneath the

skin of the back of another rabbit, in which forty-four hours previous to that the usual bladder operation had been performed. On the noon of the 23d, at 4 P. M., temperature 38; 24th, at 2:30 P. M., 39.7. Did not eat anything during the day. October 25th between 8 and 9 A. M., death ensued (43 hours after inoculation).

Autopsy at 2 P. M., October 25th. Region of inoculation shows bloody suffusions and discoloration of the muscular substance. Trachea and large bronchi, normal. Lungs pale, without presenting anything striking. Intestines, mostly empty; small intestine filled with a yellowish slime; in the ilium a very much swollen Peyer's patch was observed. Liver not particularly large. No trace of peritonitis or suppuration around the bladder. Spleen very much swollen, firm. Kidney hyperæmic.

Bladder diphtheria very beautifully developed.

Small particles inoculated into two mice, which died on the 27th October (within two days).

With this, the scarlatinal diphtheritic poison had been transplanted through two generations, and had retained its poisonous properties.

Let us again study its nature in the diseased portions. Fine sections from the bladder of Exp. xxiv show the following:

Viewed macroscopically, the diphtheria is present in little islets, so that hæmorrhagic inflammatory infiltrations alternate with coagulation-necroses. These hæmorrhagic inflammatory infiltrations still show normal epithelium in some places. In others we find them associated together with capillary hæmorrhages and infiltrations in the uppermost layers, also croupous membranes passing gradually into the normal epithelium of the still healthy portion. Within the dilated capillaries and veins (not certain without) we find accumulations of bacteria of three different kinds:

1st. Globulus bacteria or micrococci, of about 0.7 μ diameter, stained intensely in gentiana blue, or fuchsin; very pretty, especially in some of the capillary loops of the most superficial mucous layers.

2d. Larger cocci of about 1.2 μ diameter, which throughout stick together like biscuits, in twos, or chains of three and four, so that they present the appearance of bacilli of from 2 to 3 μ long, with low powers of the microscope.

Fig. 6 conveys a very good idea of such formations. Several capillaries, situated right in the midst of the diphtheritic patch, are

densely crowded with them. Fig. 6, b, shows them more highly magnified.

3d. In the larger blood-vessels which still contain blood corpuscles, a third variety of micro-organisms seem to be present, namely, bacilli of 1.8 μ in length, and 1 μ in breadth (measurement not absolutely accurate), which, however, refused to be deeply stained by any of the aniline colors, so that they were invisible under ordinary illumination, and under diffused light appeared as very minute and faint shadows. I am not quite certain whether they are a peculiar species of organisms, or whether they are simply those mentioned under the second head, very faintly stained. The former seems to me more probable.

At any rate, we have here to do with a mixed infection, such as has frequently been produced in the animal organism; and this mixed infection was transmissible from the first diphtheritic scale to the second. For even in the diphtheritic bladder of the second animal, these different species of micro-organisms were present—though, perhaps, not as abundantly, so far as the diphtheritic portions were concerned. The latter is intelligible, because inoculation was undertaken, after the disease of the mucous membrane had already progressed to the end of the second day.

These three different kinds of organisms could also be demonstrated in the kidneys of both of these animals.

So it seems pretty clearly made out, that the diseases produced in this series of experiments are very closely connected with those micro-organisms which may be regarded as identical with the poison underlying the production of those diseases. The slimy exudation in the throat in scarlatinal diphtheria also contains an inoculable poison, which rapidly increases in quantity and, perhaps, virulence within the body and produces a deadly and fatal disease, which can be transmitted, and which produced death within sixty hours in the first animal, and within forty-three hours in the second animal inoculated with it. In this instance we have again shown that the poison is especially collected and stored up in abundance in the locally diseased organ.

Thus we have really and successfully produced, per synthesis, the diphtheritic infectious disease—a disease of the animal body characterized on the one hand by a general infection, and on the other by a local process of coagulation-necrosis, and by an accumu-

lation of the poison at the place where the local disease takes place. And now we are face to face with the diphtheritic poison in the shape of these micro-organisms.

There remains, however, now to add to this, that in the human subject the taking up of these bacteria alone is sufficient to produce the disease, both local and general, while the animal mucous membrane does not respond by that peculiar inflammatory condition to the action of these micrococci. This condition must be produced artificially.

Before we consider these questions more in detail, we will first record two more experiments which were made on the diphtheritic animal of Experiment XXV. Besides the above mentioned inoculation of two mice with the diphtheritic membrane ofthe animal in question, two more inoculations were carried out as follows, on October 25th, 1882:

1. EXPERIMENT XXVII.—A strong, brown-red rabbit received, subcutaneously, a certain quantity of the patch in the right side of the back, all the necessary precautions of heating all the instruments having been observed.

2. EXPERIMENT XXVIII.—The ears of a somewhat smaller rabbit were inoculated with a bit of the patch.

Both animals became very feverish, which condition lasted six days, or until October 31st, in each one of them. The temperature, taken daily at 3:00 P. M.,was always over 40.0° C.; the highest temperature in No. 27 was 41.0° C.; in No. 28, 41.4° C. Appetite bad. In the beginning of November the temperature was again normal (39° to 39.5°). They appeared to be very well again, and No. 28, the animal which had simply been inoculated, remained entirely well. It died towards Christmas, sometime after all abnormalities due to its inoculation had disappeared.

No. 27, however, the animal which had received the subcutaneous injection, was found on November 10th with a purulent pan-ophthalmia of the right eye and a purulent discharge from the nose. The wound on the back had opened and now showed a rather extensive pus cavity. The eye gradually dried up, but the nasal catarrh remained ; the animal was often seen eating. On November 25th, it having still eaten in the morning, it was found dead in its cage in the afternoon.

Autopsy was made on the afternoon of November 26th. At the

place of the wound there is a pus cavity ; right eye dried up. The mucous surface of the soft palate and pharynx were in a condition of purulent catarrh and covered with pus. Larynx and trachea free. Purulent bronchitis in both lungs. A large number of scattered abscesses. Pleural cavities free. Heart normal. No peritonitis. Spleen, liver and kidneys apparently normal.

This last experiment is remarkable. The animal became feverish just exactly as did the other animal which had simply been inoculated ; this was regarded as the result of an infectious disease analogous to that from which animal No. 25 died, with this difference, however, that the fever was much lighter here than in the previous case, and both animals survived. But now a pus cavity formed at the end of a week at the place of inoculation and from it the pyæmic infection from which the animal died took its start. It seems very probable that this animal, on the 25th of October, received into its system two infectious poisons, one of which was the cause of the fever, the other the cause of the pyæmia, which appeared much later. It is much less probable that some extraneous poison had been introduced in the operation, for, of a number of animals operated on at the same time, this was the only one so affected.

It will be remembered that just in that infectious disease of animals 24 and 25 there were found two or three different kinds of bacteria in the blood-vessels of the diseased bladder. These different poisons must then have originated from the mouth of the child suffering with scarlatina, from which the original material for inoculating the rabbit had been taken.

But which one is now the diphtheritic poison? Are we to look upon it as the cause of the six days' fever following the inoculation ? It cannot be identical with a pyæmic poison.

With these doubts we have arrived at a question, the discussion of which has already been engaged in by many observers, but the solution of which would finally decide all experiments with infectious poisons hitherto made.[1]

Is the infectious poison which is contained in the diphtheritic patches in the throat of a man suffering with the disease, really of

1 With the single exception of the experiments of Klebs (Archiv. f. exp. Pathologie Bd. iv. s. 238), and Rosenbach (Virchow Archiv. Bd. lxx, 1877, 2 Heft), who did not take the material for the infection out of the mouth. But their results were not sufficiently clear, and their conclusions were not indisputable.

a diphtheritic nature, or does it belong simply to one of the septic poisons of which, as has been demonstrated by Koch's experiments, we have not only one but a great many?

This question, very unfavorable to the theory of the specific nature of diphtheria, could be readily answered, if it could be proven that quite similar infectious conditions could be produced by the introduction into the system of the mucous secretion of men not suffering from diphtheria, or such as are entirely well, as are produced by inoculation of such secretions from men suffering with diphtheria.

This has already been experimentally proven. After Dr. Renaud and Lanmelongue[1] had produced a transmissible infectious disease by a subcutaneous injection of saliva from a child dead with lyssa into a rabbit, Pasteur[2] found that an analogous infectious disease could be induced in the same animals by inoculating them with the saliva of children who had died with catarrhal pneumonia. Koch[3] is convinced that this infectious disease with its malignant œdema, may be induced by bacteria coming from different places. Soon after, Vulpian succeeded, by injection of saliva from healthy men into rabbits, in producing a disease from which they soon died, and in which the blood contained many micro-organisms of which several resembled those of Pasteur; and a drop of such blood, diluted with water, sufficed to produce the disease in another animal. This disease in question was, then, a genuine septicæmia.

I further find in a communication of Dr. Kühn[5] in Moringen, the following remarks: Fresh and healthy human saliva, injected into rabbits may cause septic fever and metastatic abscesses.

In order now to form for myself a correct idea of these facts, I undertook the following experiments:

EXPERIMENTS XXXIII — XXXVI.—At 3 P. M., December 27th, 1881, a turbid, slimy fluid was taken from the back part of the tongue of a perfectly healthy man, and small quantities of it were placed under the skin of the four rabits, wounding at the same time the muscular fascia; the wound was immediately sewed up.

1 Bulletin del'Académie I. m. Séance, der 1 et der 8 Fcón, 1881.
2 Ibidem Commemoration of Perrot. Séance du 22 Mars 1881.
3 Gaffky, Mittheilungen d. Kaiserliche Gesundheits Amtes Bd. i S 93 flg.
4 Bulletin etc. Séance du 29 Mars 1881.
5 Berliner Klin. Wochenschrift, 19 Sept. 1881, No. 38 S. 547.

Every one of the rabbits died between the fifth and ninth days after infection, with the following symptoms:

Rabbit No. 35.—Temperature on day of inoculation, 39° C.; December 29, 40.° C.; December 30, 40.7° C.; December 31, 40.8° C. On the morning of this day an abundant purulent nasal discharge was discovered.

During the night of December 31st the animal died.

Autopsy at 1 P. M. The skin of the whole of the right side was found undermined by an extensive abscess, containing bad-smelling, cheesy, purulent masses. Muscles at place of inoculation of a dirty red color. Spleen distinctly enlarged, brownish red, and firmer than normal; capsule moderately dense. Kidneys hyperæmic. Nothing abnormal about the liver. Bladder normal. Lungs and pleura normal.

Rabbit No. 37.—Temperature at day of inoculation, 38.9; December 29, 39.5; December 30, 39.6; December 31, 39.3; January 1, 39.6. Died during the night from the 1st to the 2d of January. Rest same as in No. 35, only more extensive. Abscess reaching from the crest of the ileum to the clavicle. Spleen not swollen.

Rabbit No. 33.—Temperature at day of inoculation 39.3° C.; December 29, 40° C.; December 30, 40.1° C. A purulent nasal discharge noticed on this day. Isolation December 31, 40.0° C.; January 1, 38.8° C. Died during the night.

Autopsy January 2d, at noon. An extensive abscess was formed, reaching up clear under the scapula. Spleen somewhat large and thickened. Trachea, lungs and pleura normal. General fibrinous purulent pericarditis. Intestines very much distended, mucous membrane strongly injected.

Rabbit No. 34.—Temperature at day of inoculation 39.4; December 29, 40.0; December 30, 41.2; Dec. 31, 40.7; January 1, 40.0; January 2, 39.5; January 3, 38.8. Found dead on January 5. Enormous pus cavity along the entire right back. In the upper left and lower right lobes of lungs some thickened hæmorrhagic spots. In both auricles and in the right ventricle large clots. Spleen scarcely swollen.

In accordance, then, with the statements of Kühn, we obtained within a very short time, as the result of the introduction of small quantities of fluid from the mouths of healthy human beings under

the skin of rabbits, extensive pus formations at the place of inoculation, and even by metastatic abscesses in the body of the animal.

An examination of the contents of the abscess showed that in all these inoculations bacteria played an important rôle, for it consisted principally of detritus and micro-organisms of various forms, as globular bacteria, scattered and in groups, and bacilli, long, short, broad, and narrow, while pus corpuscles were rare. The process was rather one of advancing necrosis than of pus formation. Groups of lymph-cells were also found in the blood of these animals. These cells contained in their immediate surroundings small granules very distinctly stained with methyl blue. It was, however, difficult to decide whether they were not Ehrlich's nuclei of mast cells.

It was not my purpose to follow out these interesting results, but they proved valuable as additional proof of the danger of saliva to rabbits, and to show that it contains bacteria which may become pathogenic in the animal organism.

But what do all these new facts tend to prove? What does it signify that inoculations with saliva sometimes produce malignant œdema, sometimes septicæmia, sometimes progressive necrosis, and sometimes pyæmic metastases in the rabbit?

This question can only be answered in one way, and that is, that in the buccal cavity of the healthy man we find various species of bacteria, which again vary in different individuals, and in the same individual at different times, both in shape as well as in their mode of action. These bacteria introduced into the circulation of certain animals, cause their death; they possess, however, no pathogenic significance for the human organism, at least not so long as they are only in the cavity of the mouth, on the most superficial layers of the epithelia.

If it is, then, certain that we find these bacteria in the cavity of the healthy mouth, how much more may we expect to find in a diseased buccal cavity, additional ones, perhaps new ones, which may perhaps have displaced the former ones, since with the disease a change in the conditions for the subsistence of the minute organisms has naturally been brought about. This is especially true of the croupous diphtheritic affection of the mucous membrane of the mouth. Here arise, in the place of the original epithelium, the uppermost strata of which certainly do not offer a very rich albuminous substratum, extended fibrinous masses, coagulated albuminous masses,

which, in the moist and warm atmosphere of the mouth cavity, offer a very different soil for the development of these micro-organisms. The fact that in such a soil bacteria may arise, which are different from those occurring in the normal cavity of the mouth, is not strange. It is not less easily understood that such new species, when inoculated into the proper animals, give rise to different forms of infective diseases than are procured by inoculation of normal saliva, that perhaps even two or three different disease processes may be inaugurated in the same animal (as I obtained on inoculation with mucus from the scarlatina patient).

Thus, we are now in a position to state positively, that these infections or inoculations with bacilli, as I obtained them from the slimy masses of primary diphtheria as well as from the mouth of the scarlatina patient, stand in no relation whatever to the diphtheria or scarlatina, as they occur in human subjects; these organisms were simply accidental, and happened to find a good soil for their development in the changed mucous membrane.

All experiments hitherto made with the diphtheritic secretion from the mouth of persons sick with the disease have, therefore, lost a great deal of their original value. No one is capable to declare, or justified in asserting that he has, in the manner above described, inoculated either scarlatina or diphtheria from one animal into another, no matter where the inoculation was made, whether in the trachea or in the subcutaneous cellular tissue, and no matter what was the course and character which the disease thus produced took on.

One cannot deny that these deductions are quite justifiable. But they are not entirely free from objections, inasmuch as they rest on a proof of simple probability. One may admit that in a healthy mouth cavity a number of bacteria accrue, which have nothing to do with the poison of diphtheria, but which are rather of a septic character. But, does this disprove the possible fact that, side by side with these septic bacteria in human diphtheria, there is the real, genuine micro-organism of diphtheria? And is it not at least very probable that it is just that short bacillus, which from the surface of the diphtheritic membrane is so easily transportable (retaining its form) to the animals, and whose shape distinguishes it so well from that of other animals, that this is not the septic bacillus but the true representative of the diphtheritic poison? Certainly we cannot

deny the possibility of the presence of the representative diphtheritic poison among the numerous species of bacteria which have been described as occurring in the mouth of those suffering with diphtheria, and this possibility even my experiments are unable to disprove; it only remains to be added that this peculiar micro-organism has not yet been identified, and that with the methods at present at our command it cannot be identified.

Then the second question, if perhaps that short bacillus be the representative bacillus to which the diphtheritic disease was due, can be answered in the negative both as a result of my experiments and as a deduction from the following line of thought.

The poisonous effect which this bacillus produces, when inoculated in the animal, is due to its enormous increase in the blood. This is simply and distinctly shown in the fact that in all cases of death it can be found in vascular districts of the most different kinds, far from the place of inoculation, but more copiously in the blood-vessels of the diseased mucous membranes.

But in those places the presence of these micro-organisms is easily proven by means of our methods of staining.

If, then, this poison was identical with that of human diphtheria, the general diphtheritic infection in the human subject would have to be represented as due to a copious and abundant increase of this bacillus in the blood, no matter how it got there, and that thereby life was destroyed. And here also would we expect to find a particularly abundant accumulation of bacilli in the diseased mucous membrane.

Consequently, in those cases of diphtheria in man, where death was not due to the mechanical effects of the disease (suffocation) but to the general infection (collapse, asthenia, paralysis of the heart, nephritis), the blood-vessels of the diseased mucous membrane, the capillaries of kidney and liver, etc., ought to be filled with bacilli.

The presence of these bacilli in the human system ought to be capable of proof, according to the same method, and in as easy and positive a manner as this is done in animals in every respect.

In this we do not succeed. Entire series of sections of the uvula, of the throat, kidneys, etc., of patients dead of diphtheritic infection, have been treated with gentiana violet, fuchsin, Bismarck brown (also in alkaline dyes), without succeeding in dis-

covering a single bacterium, which, on the diseased surface of the mucous membrane, are so easily made out. Consequently the diphtheritic poison has nothing to do with the so-called "pilzrasen" (a much abused and often cited term) which covers the diseased mucous membrane of the cavity of the mouth.

Therefore, then, the diphtheritic poison is as yet unknown, as are numerous other disease-poisons, *e. g.* that of scarlatina, of measles, of small-pox, etc. The diseases I produced in my inoculation experiments, were, it is true, diphtheritic, in so far as they for the first time represented undoubted combinations of a local diphtheritic disease of the mucous membrane and a general infectious disease; but still the infectious poisons used were not identical with the poison in human diphtheria, but were entirely of a heterogeneous nature.

I do not hesitate to acknowledge this negative result, to whicn my second series of experiments and the train of thought to which these gave rise, have led me, for it is not a step in the wrong direction in the search for truth, to recognize an error and to publicly acknowledge it. We do not, therefore, give up hope that the diphtheritic virus may not some day be discovered, it will, in all probability, require entirely new methods in order to be successful in such a search.

In the case that the general infection in diphtheria be produced, as is very probable, by micro-organisms increasing rapidly in the blood, then all of our attention would have to be directed to the diseased blood vessels of the affected mucous membrane and to their contents. It would be there where we would have to look for an accumulation of the poison, according to my experiments.

For those who are convinced that the poison of diphtheria is even now not discovered, another question remains to be answered, and a question which, by many physicians, is generally looked upon as settled, namely, whether the poison first finds a place on the surface of the mucous membrane, and thence makes its way into the blood, or whether the diseased mucous membrane is a secondary localization which follows a primary infection of the blood. From my experiments on the condition for the local diphtheritic process in the mucous membrane, it follows that the profound change in the tissues underlying it all might just as well be produced by some noxious agent circulating in the blood as by one

finding its way from the surface of the mucous membrane into the interior.[1] I can add nothing more for the solution of this important problem from my experimental research, and cannot therefore go any further.

1 As analogies we may cite the diphtheritic patches which arise in some places on mucous membranes after injections of corrosive sublimate or arsenic.

EXPLANATION OF PLATE.

Fig. 1.—Formation of hæmorrhagic œdema and diphtheritic patch in mucous membrane of bladder of rabbit.

The picture represents a fine section from the fundus of a rabbit's bladder which had been subjected to treatment with the ligature, as was described in the text, 48 hours before the animal was killed.

Stained by eosin and gentiana-violet.

Magnified—Zeiss CC. Oc. I.

The portion on the right is in a condition of hæmorrhagic œdema; only part of the much swollen submucosa is visible and, within it, several engorged blood-vessels. The epithelium is much swollen up and œdematous, and, as the mucosa, studded with extravasated blood corpuscles.

The portion on the left is the diphtheritic eschar; epithelium, mucosa, submucosa, cells, vessels, etc., have become changed into a homogeneous mass, stained red with eosin, within which only a small amount of nuclear detritus (stained blue) is to be seen.

Fig. 2.—Beginning coagulation-necrosis of the epithelium. From the bladder of a rabbit, killed 24 hours after the removal of the ligature. Boiled preparation. Stained with eosin and gentiana-violet. Mag. Zeiss F. Oct. I. 1-410 (not 1-490 as it says on plate.)

Only portion sharply focussed is the one in which the exudation has thoroughly penetrated the epithelia. Some of the cells, whose nuclei have ceased to be stainable, are pressed apart by the coagulated exudation, which latter has taken on a deep-red stain.

Fig. 3.—Croup of mucous membrane of bladder.

The rabbit from which this specimen was taken was killed six days after the removal of the ligature.

Staining by gentiana-violet. Magn, Zeiss CC. Oc. I.

One sees a fragment of croupous membrane still adhering to the underlying mucous tissue. The limit is marked by the blue stain which the mucous tissue has taken up and which has arisen from a very dense infiltration of nuclear detritus (not of normal lymph cells.)

Within the croupous membrane numerous nuclei of emigrated cells.

Epithelium is missing throughout.

Fig. 4.—Diphtheritic eschar, within which blood-vessels, still pervious, are found.

Animal killed three days after removal of ligature, at the end of the second day, had been inoculated with bacillus anthrax, from which inoculation it died towards the end of another day. The swelling of the diphtheritic portion of the mucous membrane was very great and intensely hæmorrhagic. Post mortem was made immediately after death and the bladder put into absolute alcohol.

Staining with eosin and gentiana-violet. Magn. Zeiss CC. Oc. IV. The staining by eosin on the slide is much deeper than is represented in the picture. Nothing more can be seen of the original mucous tissue

(this portion, two days earlier, would have presented the appearances of the right half in Fig. 1).

The portion included between the violet line and the adjoining brownish-red line, is the transformed epithelial layer; all the rest is mucous and submucous tissue that has undergone coagulation-necrosis. The brownish-red stained portion has originated from the hæmorrhagic part at the diphtheritic eschar. In that slightly granular homogeneous tissue a few nuclei from emigrated cells may be seen.

In the midst of this eschar several open channels filled with bacilli anthracis are seen. The greater portion of the walls of these channels is stained red; they are without nuclei, and may be looked upon as dead. Some nuclei are, however, visible in the wall of the larger vessels on the right—an arrangement which marks this vessel as a small artery.

In all these dead blood-vessels, circulation must have been still going on a few hours before death took place, as may be supposed, from the abundant presence of bacilli in them.

Fig. 5.—Accumulations of micro-organisms (from the mouth of a diphtheritic patient) in the capillaries of the diseased bladder.

Section from the bladder of a rabbit which had been inoculated with the slime from a diphtheritic tonsil, and in which death occurred three days after the inoculation; a portion is chosen which shows the epithelium in a condition of hæmorrhagic œdema. Illumination with Abbe's condenser without diaphragm.

Staining with Fuchsin. Magn. Zeiss J. Oc. II.

Two capillaries are seen densely crowded with small bacilli.

Fig. 5.—Shows these bacilli on a more enlarged scale (Ziess J. Oc. IV).

Fig. 6.—Accumulations of micro-organisms (from the mouth of a scarlatina-diphtheritic patient) in the capillaries of a diphtheritic eschar of the urinary bladder.

Section from a diphtheritic patch of the bladder of a rabbit which had been inoculated with slime from a scarlatina-diphtheritic patient, and which died three and one-half days after that inoculation.

Illumination and focussing same as in previous preparation.

Staining with Fuchsin; in the preparation more violet than blue. Magn. Zeiss J. Oc. II.

Capillaries crowded with micro-organisms, still visible within the patch.

Fig. 6.—Shows that these seeming bacilli are micrococci, arranged in pairs or chains.

O. HEUBNER, Diphtherie.

Fig. 1.

1/410.

Fig. 2.

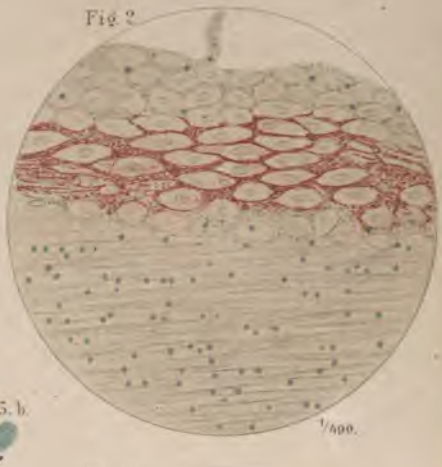

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Fig. 3.

1/110

Fig. 3. b.

1/1090

Fig. 4.

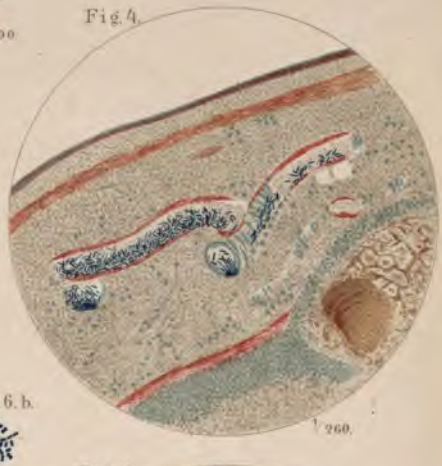

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Fig. 5.

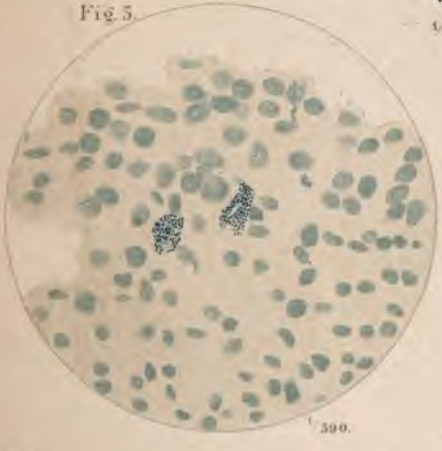

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Fig. 6. b.

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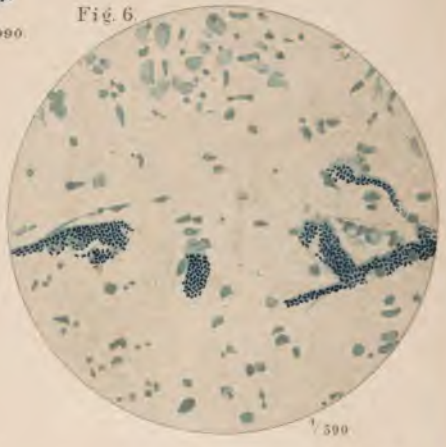
Fig. 6.



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APPENDIX.

This appendix embraces, in abstract form, a paper on diphtheria, read by Dr. Gerhardt, of Würzburg, before the German Medical Congress, which held its session last year in Weisbaden, and also the discussion on diphtheria which followed, and which was entered into by Klebs, Heubner and all the prominent delegates there.

Dr. Gerhardt.—Diphtheria is an infectious disease. Abundant proof of this has been produced both clinically and experimentally. The poison of diphtheria has, moreover, the property of remaining alive and effectual for a certain length of time, at ordinary temperature, outside the animal body, and can be carried in clothing and adhere to houses and the walls of rooms. Observations are on record apparently tending to prove that the diphtheritic poison has gained entrance into the human body through food, (milk, etc.) The poison is furthermore transmissible from man to animals, and Dammann has described a diphtheria among calves; and perhaps also from animals to man as Dr. G. seems inclined to believe from the following remarkable circumstance which was brought to his notice:

"In the village of Messelhausen, near Landa, in Baden, a chicken farm had been started, into which 2,600 chickens had been brought from the country near Verona, Italy. A few of these had diphtheria and, within the first six weeks 600 of them died of the disease, and later on 800 more. The following summer 1,000 chickens were hatched from eggs laid by these hens and all of these died of diphtheria within the first six weeks. Five cats succumbed to the same disease at this farm, and a parrot also took the disease but was saved. In November, 1881, an Italian rooster, about to be touched up with carbolic acid, bit one of the attendants in the left hand and foot. The man was taken sick with high fever and both wounds were covered with diphtheritic membranes. The wounds healed very slowly, the disease lasting in all three weeks. Two-thirds of all the farm hands became affected with diphtheria

of the throat, and pharynx and, at the same time, not a single case occurred in the adjoining village."

Diphtheria may be said to be always accompanied by fever, or at least those cases in which no fever is present are exceedingly rare. This fever differs in many respects from that accompanying other infectious diseases. Thus we find that in many infectious diseases as in variola, measles, etc., the original fever is caused by a peculiar process of infection which takes place in the blood, and which is characteristic of these diseases; and that, on the contrary, certain periods of fever which supervene later on, as for instance, the fever of suppuration, the fever of decrustation in variola, those forms of high fever occurring in parotitis when the testicles become implicated, are independent of the original fever. It is very different with diphtheria. Here the fever is directly dependent upon the local affection and the rule may as well be stated here that, as long as the local affection keeps on increasing the fever will also increase, and that as soon as this ceases the fever will also cease.

Glandular swelling in diphtheria is directly dependent on the passage of the poison into the lymphatics and into the economy at large, and its traces may, moreover, be discovered in the subsequent rheumatoid affections, in nephritis, endocarditis, peripheral nerve-lesions and other allied affections. Into this category also belongs that peculiar form of anæmia, characterized by great feebleness of the heart's action with great increase in the pulse rate, which by some is looked upon as a paralysis of the vagus, and by others is interpreted as a fatty degeneration of the heart.

In diphtheria it is especially necessary to differentiate, and the microscope will, no doubt, prove the best means of doing this, for with it we can discover the infectious agent. But, since the time has not yet arrived when every physician is expert enough to make this sort of differentiation applicable to all cases, it would be premature to recommend it.

So far as age is concerned, although diphtheria may attack new-born infants, the first year of life is almost exempt from the disease; it is most liable to appear between the first and fifth year of life. Moreover, diphtheria has a stage of incubation of an indefinite length of time; according to H. Roger, it is between two and seven days, according to others, it may be fourteen days. One of the most

important features, according to Dr. G., is that the stage of incubation is indefinite as to length of time. He calls attention to the fact, that certain infectious diseases, more especially those which terminate in a crisis, also have a stage of incubation of a definite length of time, as, for instance, typhus, measles, variola; and that certain others, in which recovery takes place by lysis, have a stage of incubation of an indefinite length of time, such as typhoid and scarlatina; he seems to be inclined to believe that this stage of incubation and mode of recovery stand in direct relation to the origin, growth and decay of the organisms which cause these diseases. Diphtheria belongs to that class of infectious diseases which have an incubation of indefinite duration, as well as an indefinite febrile stage, and which terminate in lysis.

Diphtheria is characterized as an infectious disease, not only in a general sense, but also from the fact that certain micro-organisms have been discovered in connection with it. Observations of this kind have been made by a great many observers, such as Tommasi, Letzerich and others. Nor is it difficult in the least to discover micro-organisms in diphtheritic membranes; anyone who has given the subject his serious attention, must have succeeded in convincing himself of this fact, and acknowledge that the real difficulty lies in the great mass of them, and in the decision as to which one of these organisms is the primary cause of the disease. Every writer on the subject believes his own to be the right one; others have come to the conclusion there must be several of them coöperating to produce diphtheria. This is not at all unlikely, and it would require no great stretch of the imagination to think that several forms of micro-organisms might be causative of diphtheria. Indeed, in consideration of the multiplicity of forms of diphtheria occurring in everyone's practice, we are almost forced into the belief that there must be several distinct varieties of micro-organisms which cause the disease, and that the different forms, and the different degrees of severity, of cases of diphtheria are directly dependent on the different forms or combinations of forms of micro-organisms.

Diphtheria becomes more especially localized on the gullet and throat, and this predilection for the mucous membrane in this situation alone, would lead us to expect certain anatomical conditions in these situations which are peculiarly favorable to such localization. Ph. Stöhr, of Würzburg, from some investigations on this

subject, has shown that, on the surface of the tonsils, the epithelia show cracks or minute loop-holes through which round-cells emigrate. If it is true, as has been demonstrated in a very convincing manner by Dr. Rühle, that the stratum corneum of the skin and mucous membrane presents an impenetrable barrier to the passage of micro-organisms, the above anatomical peculiarities of the mucous membrane of the tonsils seems to create an exception to this rule.

According to what has been said, it will be seen that Dr. Gerhardt shares the belief that diphtheria is caused by micro-organisms, and that they find their way into the economy principally through mucous membrane of the throat, though other channels may be open to them. The treatment which he principally recommends may, therefore, be anticipated, and consists mainly in the attempt to dissolve away the membrane and thoroughly disinfect the throat. He, however, speaks discouragingly of the application of anything that is irritating to the throat and which may produce inflammation, for inflammatory foci present a condition of things which is always favorable to the implantation of micrococci. Little weight, if any, is laid upon constitutional treatment.

Klebs expresses himself as extraordinarily pleased with the clinical results obtained by Dr. Gerhardt, and states that he has arrived at almost the same conclusions, although in an entirely different manner. This has reference, more especially, to the composite character of diphtheria. He then enumerates various disease-processes which are accompanied by the promotion of fibrinous membranes, such as dysentery, diphtheria of the bladder, croupous affections of the lungs, which are non-infectious, and, therefore, to be excluded from under the term diphtheria. The researches of Oertel, Eberth, and himself, have sufficiently demonstrated the infectious nature of genuine diphtheritic membranes and their transmissibility to the cornea. From extensive examination of membranes from cases of diphtheria met with in Prague, and which were characterized by great gravity and prominent nervous symptoms, with hemorrhagic formations on brain and spinal cord on post-mortem examinations, he obtained specimens, some of which were exhibited, in which some very peculiar micro-organisms were found. From the fact that they were transmissible to the cornea, he concluded that they were the organisms to which the disease was due and designated them as the "microsporon diphtheriticum," which, like microsporon

septicum, have the peculiarity of showing two different stages of development, that of bacilli and round, globular micrococci.

Later on, however, in Zürich, cases of diphtheria occurred which were evidently of an entirely different character from those that had come under his observation in Prague. The membrane of the throat, in these cases, had a great tendency to extend into the trachea, and life was generally terminated in that way, but also, sometimes, through collapse. These cases were also found to be accompanied by albuminous urine, and disease of the kidneys was suspected, but the suspicion was not sustained for the reason that the symptoms of uræmia were wanting. Search was made for micro-organisms and they were found, but of an entirely different nature from those found in former cases, namely, instead of their being globular, they were exclusively bacillar formations. The two forms of diphtheria which Klebs distinguishes, had their origin in this way. The one is the diphtheria micro-sporon, and the other the diphtheria bacillaris.

As further anatomical changes which are peculiar to the bacillar form of diphtheria, he gives the following: Only very light enlargement of the spleen, if any; no parenchymatous degeneration of the kidneys, and no changes in the heart and liver; those dying from it generally do so from severe lung affection. The microscopical examination of such lungs generally shows very extensive interstitial deposits : interstitial diphtheritic pneumonia.

This lung affection generally takes on a chronic course, and keeps on long after the diphtheria has entirely disappeared.

The characteristics of the bacillar diphtheria are summarized as follows :

1. Fibrinous exudation on tonsils, with great tendency to extend into the trachea.

2. Constant presence of a definite form of bacilli on the diseased mucous membrane, which are primarily developed within the epithelial cells, and giving rise to the fibrinous exudation as a consequence of a peculiar paralysis of the blood-vessels.

3. High fever, which accompanies the extension of the fibrinous exudation but which ceases later on, and only recurs on the occurrence of septic infection.

4. Interstitial inflammatory processes in internal organs, as sequelæ, as it seems, not, however, as much dependent on the pres-

ence of micro-organisms within these organs, as on extensive changes taking place in the blood-vessels (interstitial pneumonia, myocarditis, neuritis, albuminuria, and interstitial nephritis).

5. Necrotic decomposition of the membrane leads to septic complications which may cause death long after the diphtheritic process in the throat has come to an end.

Heubner.—The experimental study of diphtheria has evidently to deal with two problems. The first is that of the peculiar local affection of human diphtheria, and the second is that of the general infection. No experiment can be called complete that does not fully answer to these requirements. The attempt to produce both these conditions by one experimental manœuvre did not seem to be successful. I, therefore, attempted to deal with these two problems separately, by first trying to produce the local disease, and by superadding to this, later on, the general constitutional part of the disease in question.

By thus producing an artificial diphtheria by synthesis, in animals, no doubt there was a strong possibility that some advance in our knowledge of human diphtheria might be made.

The method of inducing the local disease which had hitherto been in practice, and which consisted in the application of caustics to mucous surfaces, impressed me as being too crude. While engaged in the study of the investigations of Cohnheim and Litten, it occurred to me that it might be possible that those severe inflammatory processes by which certain tissues, the testicle and kidneys, responded to a temporary interruption of the blood current, might also be produced in the mucous membrane, and these might prove to be morphologically identical with diphtheria. This hypothesis was afterwards confirmed, as soon as the proper mucous tissue had been found, in which such a temporary interruption to the blood current could be brought about. The mucous membrane of the throat could not be made use of, for neither dogs nor cats possess uvulas. After looking around a good deal it was found that the urinary bladder of the rabbit proved to be the best object for experimentation, and in which a temporary arrest of the blood-current could well be brought about. It was comparatively easy to cause a sudden arrest of the blood current, and maintain it by a ligature around the fundus, and above the entrance of the ureters, which ligature could be removed again very easily and the circulation

be allowed to be resumed, the blood made to re-enter the area of vessels thus treated.

After such an operation it was found that, during the first day, the mucous tissue became hæmorrhagico-œdematous; during the second day this fluid œdema began to coagulate, and on the third day a picture, identical with that of diphtheria, presented itself and could easily be recognized.

It is, therefore, very evident, after a careful study of the conditions which are causative of this local disease, that Cohnheim's postulate is hereby fully confirmed, namely, that it is the result of a supervention of necrosis upon inflammation. The moment the epithelium looses its nuclei, coagulation within and upon the mucous tissue begins to set in. These two conditions, then, must be considered as the causative ones of the local part of diphtheria.

In order, now, to dispose of the second problem, an infectious disease was produced in an animal whose bladder had already been rendered locally diphtheritic, and in order to facilitate study on this point, the poison of anthrax, so easily recognized, was employed. Animals were inoculated at different periods of the progress of the development of the local disease, so that its influence might be studied early as well as late in relation to the local disease.

The anthrax poison accumulated in those locally diseased tissues when inoculation was made early. In the earlier stages of the inflammation the bacilli anthracis almost completely filled the capillaries, much more so than usual, but the poison did not leave the blood-vessels. Heubner has never succeeded in convincing himself of these bacilli ever showing themselves outside the capillaries.

After having found that the infectious agent in the presence of the locally diseased mucous membrane accumulates in the enlarged blood-vessels, instead of taking the anthrax poison, diphtheritic masses were now inoculated just in the same manner as had been done by Oertel.

A portion of the slime, rich in bacilli and micrococci, from the tonsil of a diphtheritic patient, was taken and introduced into an animal which had already been rendered locally diphtheritic. This case showed very plainly that there is no doubt of an acute infectious disease being produced in an animal inoculated with such material. The disease is characterized by an enlargement of the spleen, hæmorrhages on serous membranes, and death ensues in from

two-and-one-half to three days. Short bacilli were seen filling the diseased blood capillaries, especially in those parts in which the diphtheria had not yet induced death of tissue.

It became evident that these bacilli really represented the poison which had caused the acute infectious disease, from the fact that portions of the membrane thus rendered diphtheritic, reinoculated into other animals, regularly caused the disease through many generations.

The second form of diphtheria had thus been produced, which taught us that—given a local disease—the poison causing the acute general infectious disease, accumulated in the locality which had, primarily, been rendered diphtheritic. Why not, then, take it as proven, that these bacilli were really the diphtheritic poison.

Heubner does not think that the evidence upon which to base such a belief is as yet sufficiently strong, and in this regard differs from the views advanced by Klebs, for the following reasons: If these micrococci or bacilli be the diphtheritic poison, then it would follow that in at least those cases of diphtheria in which death is caused, not by the local disease, but by the acute general infectious disease, these bacilli must needs have been present, such presence being liable of proof with the same methods in the locally diseased blood-vessels as in the artificially produced diphtheria. Entire series of sections of uvulas have been made, and while micrococci could be plainly recognized on the surface, none were seen within the lumen of the otherwise enlarged blood-vessels, and thereupon the conclusion *that the organized materies morbi of diphtheria has, up to date, not yet been found.* Nor does H. consider it proven, that the diseased mucous surface is the locality where the diphtheritic poison first establishes itself and that thence it finds its way into the general economy. As a further reason for his views he adduces the fact that no case is hitherto known of an infectious poison penetrating through healthy epithelium. The attempt to introduce infectious material through the mucous surface of the vagina of the rabbit has never succeeded. In all our infectious diseases depending on bacteria, the latter enter the economy either through inhalation, or food, or inoculation, and not through healthy epithelium. Then, with regard to the relation of the local process of diphtheria to the fever, we find that the highest fever is always found on the first day of the disease, and regardless of the spread of the local trouble. Such is certainly not

the case in an infectious disease depending on local infection. In erysipelas, for instance, we have a new rise in temperature for every new spread of the local disease.

As a third reason he gives one based on therapeutical results. In his opinion the local treatment, beyond a certain amount of cleanliness, amounts to nothing, and that it is through internal treatment that we must try to antagonize the poison.

In this respect Heubner agrees with the great majority of practical American physicians, as will be readily seen by reference to the "*Collective Investigation of Diphtheria as Conducted by the Therapeutic Gazette, Detroit, Mich.*" Out of 92 physicians who gave a positive opinion with regard to the treatment of the disease, only 12 advocated or laid more stress on local treatment than on constitutional treatment; 29 declared themselves decidedly for constitutional treatment alone and 51 for both local and constitutional, laying, however, the greatest weight on the constitutional part of the treatment.

Jürgensen believes in a very thorough local disinfection in all cases of diphtheria, but not with caustics; he is of the opinion that a general disinfection of the body is not practicable and, that the only thing to be done in the way of general treatment is, to supply nourishment in abundance.

Rossbach cannot agree with Heubner with regard to the penetration of micro-organisms through the mucous membrane of the throat. The tonsils, he argues, are organs through which constantly large numbers of white blood corpuscles emigrate, and therefore he thinks that wherever a white blood corpuscle can come out, a bacillus may get in. He tells of cases of diphtheria in which the tonsils had previously been removed and in which the places formerly occupied by the tonsils remained free from the diphtheritic invasion. He recommends papayotin for the purpose of digesting the false membrane in diphtheria, but at the same time calls attention to the fact that there is papayotin in the market which has lost all digestive powers and is useless, and that, therefore, care must be taken to get a good preparation.

Leube, notwithstanding the general prejudice against the application of any caustics to the throat in cases of diphtheria, admits having obtained excellent results from an application of a solution consisting of carbolic acid and alcohol in equal proportions. He says the application, in order to prove effectual, must be made

early and thoroughly. Besides trying this on a great many other patients, L. has had the opportunity of trying it on himself twice and always with great success. Once he was taken with the disease and his tonsils were completely covered with the exudation within a few hours. One single but thorough application of the solution of carbolic acid and alcohol was made. Towards evening the fever went down and on the next morning the exudation in the throat had entirely disappeared. In the second case occurring on his own person, three applications became necessary, but that was the highest number of applications he ever found it necessary to make. Leube is somewhat inclined to believe in the chemical nature of the diphtheritic poison, though he is very guarded in how he expresses this belief. This view certainly finds support in the fact that albuminuria, which is usually present in diphtheria, is not directly caused by the presence of micro-organisms within the kidney, and examination of the urine has never resulted in the finding of bacilli or other micro-organisms in cases of diphtheria. On the other hand, the bacillus tuberculosis has been found in the urine in cases in which the disease had made considerable progress in the kidney.

Edlefsen states that his experience had always led him to believe that scarlatinal diphtheria was something separate and distinct, and that this kind of diphtheria never extends to the throat and trachea. He admits, however, that a complication with true diphtheria may obtain in scarlatina. Concerning the cause of death in diphtheria directly traceable to disease of the heart, he remarks that this heart complication is not always due to a parenchymatous, fatty or granular degeneration of the heart-muscle, but sometimes, also, to a paralysis of the nervous apparatus, more especially in the heart ganglia. He does not think that the trouble in these cases is due to paralysis of the vagus, since the characteristic point is a slowing of the pulse-rate, which contrasts very markedly with the acceleration we meet with in muscular degeneration.

Lichtheim maintains that all those diseases which have been produced by the inoculation with micro-organisms, taken from the throat of diphtheritic patients, were not diphtheria and had nothing to do with diphtheria; these micro-organisms were simply accidental parasites, and the diphtheritic virus remained still to be discovered.

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